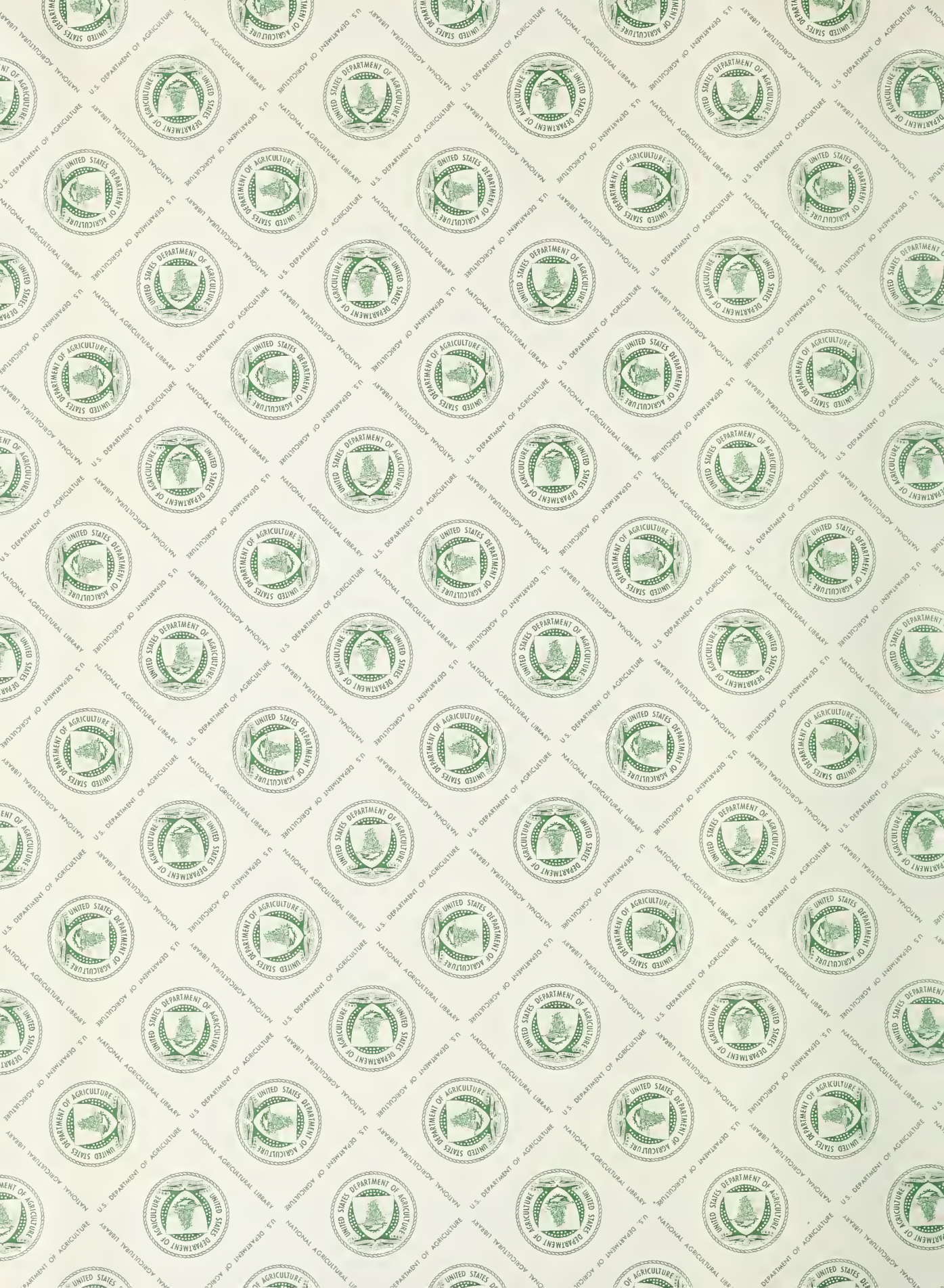
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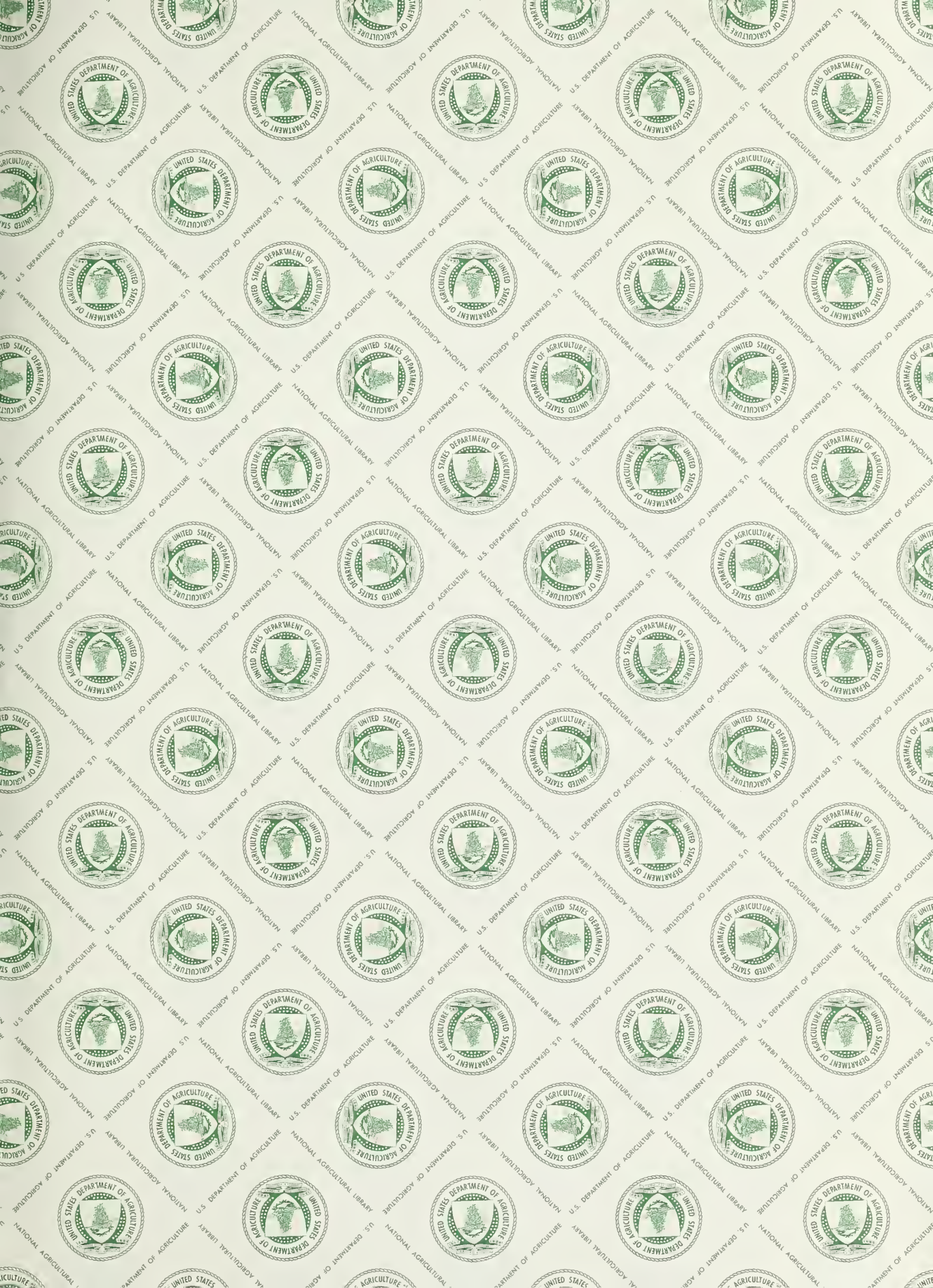
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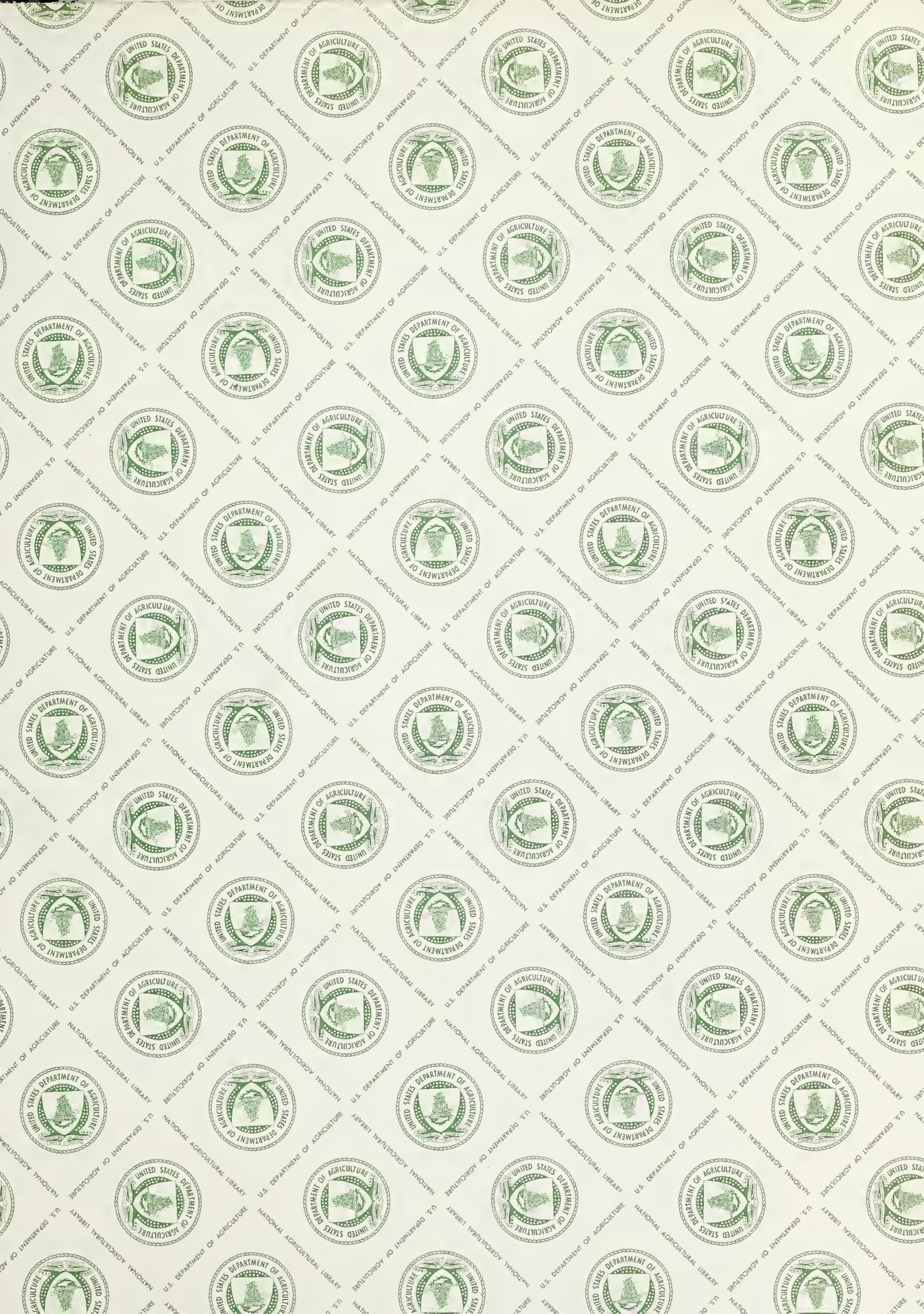
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A Summary of Current Program 7/1/66

and Preliminary Report of Progress

for 7/1/65 to 6/30/66

ANIMAL DISEASE AND PARASITE

RESEARCH DIVISION

of the

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

and related work of the

STATE AGRICULTURAL EXPERIMENT STATIONS

This progress report is primarily a tool for use of scientists and administrators in program coordination, development and evaluation, and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on USDA and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of USDA and cooperative research issued between July 1, 1965, and June 30, 1966. Current agricultural research findings are also published in the monthly USDA publication, Agricultural Research. This progress report was compiled in the Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.

UNITED STATES DEPARTMENT OF AGRICULTURE

Washington, D. C.

July 1, 1966

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INTRODUCTION

The Animal Disease and Parasite Research Division administers a national program of basic and applied research on diseases of cattle, poultry, swine, sheep, horses, and fur-bearing animals. The Division consists of three large laboratories and eleven smaller, specialized laboratories. The large ones are the Beltsville Parasitological Laboratory, the National Animal Disease Laboratory, Ames, Iowa, and the Plum Island Animal Disease Laboratory at Greenport, Long Island, New York. The research at these locations covers, respectively, animal parasites, animal diseases existing in the United States, and foreign animal diseases. The smaller, specialized laboratories are located as follows:

Southeast Poultry Research Laboratory, Athens, Georgia.
Regional Animal Parasite Laboratory, Auburn, Alabama, with substation at Experiment, Georgia.
Endoparasite Vector Pioneering Research Laboratory, Pullman, Washington.
Toxicological Research Laboratory, Kerrville, Texas.
Ectoparasite Vector Research Laboratory, Denver, Colorado.
Poisonous Plants Research Laboratory, Logan, Utah.
Parasite Research Laboratory, Albuquerque, New Mexico.
Parasite Research Laboratory, University Park, New Mexico.
Swine Parasite Research Laboratory, Tifton, Georgia.
Cooperative Research at the State Veterinary Research Institute, Amsterdam, Holland.
Cooperative Research at the East African Veterinary Research Organization, Kabete, Kikuyu, Kenya, East Africa.

In addition, the Division engages in other research involving forty-three cooperative projects and research contracts at various universities and State Experiment Stations. The Division's research program is coordinated by the Office of the Director, located at Beltsville, Maryland.

The Animal Disease and Parasite Research Division has contributed many significant research findings aimed at reducing the heavy losses to the livestock industry resulting from animal diseases. Several of these research discoveries have accounted for savings to the livestock industry in excess of the total cost of animal disease research in the U. S. Department of Agriculture since the inception of the Bureau of Animal Industry in 1887. Among these discoveries are the isolation and description of the genus of bacteria known as Salmonella; the role of arthropod vectors in spreading infectious diseases; the cause of hog cholera and the development of the first immunization procedure for this disease; the first successful treatment for hookworms in animals and man; the development of strain 19 vaccine to prevent brucellosis, and the discovery of the cause of hyperkeratosis in cattle. Some of the more recent accomplishments by this Division are -

Replication of bluetongue virus in the salivary glands of an insect vector, Culicoides variipennis. Bluetongue virus was found to multiply in the salivary gland tissue of Culicoides variipennis, a known vector of the disease. This is the first time that an arbovirus affecting animals has been demonstrated in the insect vector. The virus was shown to develop in close association with an unknown structure presumed to be ribonucleoprotein. This finding is an important first step in establishing how insects transmit virus disease, which must be thoroughly understood before eradication or control of arboviruses may be realized.

New structural characteristics of the causal agent of bovine anaplasmosis discovered by electron microscopy. Newly devised electron microscope techniques revealed that the anaplasm in the red blood cells of cattle contains ultrastructures like those of protozoan parasites. This new finding is important because the biological nature of the anaplasm has been unknown and methods of control and eradication have been hampered thereby. Many investigators have contended that the anaplasm belongs to a class of microscopic organism known as rickettsia and measures against the organism have been formulated on that basis. Evidence that the organism is a protozoan will make possible a more rational approach to treatment, control, and other aspects of the problem. Anaplasmosis costs cattle producers in the United States in excess of $25 to $50 million annually.

Development of new methods results in advances in cultivation of parasitic nematodes. Cultures of swine kidney and liver cells combined with enriched tissue culture media have been used to obtain advanced development of Stephanurus dentatus and Ascaris lumbricoides, the most damaging roundworm parasites of swine. Advanced stages of these nematodes, heretofore available only in small quantities from slaughtered animals, can now be grown in quantity for use in investigations on biological control.

Swine stomach worm found to overwinter in dung beetle grubs. Infective forms of the stomach worm of swine Physocephalus sexulatus have been found to overwinter in the grubs of the dung beetle, Phanaeus vindex where they are well protected. This finding throws new light on the perpetuation of this important parasite of swine.

Effect of anti-viral substances on a transmissible gastroenteritis virus. The effect on the plaque production of a cytopathogenic virus from transmissible gastroenteritis of swine by 5-bromo-2'-deoxyuridine (BUDR), 5-iodo-2'-deoxuridine (IUDR), actinomycin-D, puromycin, and amantadine-HCl (Symmetral) have been studied. Symmetral reduced the plaque-forming units of virus per ml by approximately 98%. Puromycin prevented almost all virus reproduction while actinomycin-D caused approximately a 22% reduction. Both IUDR and BUDR produced approximately a 20% increase in plaque-forming units of virus per ml. These findings provide an important lead toward the development of anti-viral substances.

Pathogenicity of psittacosis. Studies on the pathogenicity of avian and mammalian psittacosis agents for wild and domestic birds and mammals were extended to include psittacosis strains isolated from cattle with encephalomyelitis or from cows that had aborted.

The significant findings of these studies were that one cattle strain was identical to psittacosis agents commonly found in pigeons, in terms of the strain's pathogenicity for various animals. A second strain from cattle was similar to that found as a cause of arthritis in sheep. This work suggests that cattle may be subject to infection with psittacosis organisms from hitherto unrecognized reservoir animals.

Herbicide safety. The herbicides most commonly used in the United States - 2,4-D and 2,4,5-T - have been shown to be of extremely low toxicity to livestock. In addition, these compounds are rapidly excreted and leave only extremely small tissue residues. Use of these herbicides on range and forage plants should offer no significant hazard to livestock.

Insecticide safety. Certain insecticides may not offer a hazard to livestock simply because they are repulsive in taste or odor. Demeton (Systox) treated forage is consistently refused by sheep, even when they are starved. It is more than likely that a thorough investigation of the repulsiveness would lead to the identification of innocuous substances that could be added to other insecticides, thereby insuring that treated forage would not be consumed.

Poisonous plants cause congenital deformities in livestock. Veratrum, lupine, and loco weeds, common poisonous plants on range areas, have all been shown to cause congenital malformations in livestock, which have been looked upon in the past years as being hereditary in origin. The type of deformity is directly related to the stage of the development of the fetus at the time the plant is ingested by the mother. Losses from such deformities have ranged from 1% to 33% of the lambs or calves born on individual ranches in which the cattle and sheep are allowed to graze open range areas. Since the cause for a number of congenital deformities has been found, it is now possible to prevent many of the malformations commonly found in livestock by breeding the animals at a time so that the fetus will not be susceptible to the poisonous plants when ingested by the mother; or, a change in management practices to graze the areas when the plant is least toxic.

Utilization of methane by the ruminant. Using radioactive carbon-labeled carbon dioxide and methane, it was demonstrated that considerable quantities of these two gases were absorbed from the lungs following eructation. It has been shown that appreciable amounts of methane are absorbed and oxidized following intravascular introduction of methane. Radioactivity has been demonstrated principally in simple carbohydrates of liver tissue of sheep. This is the first work showing that mammalian tissue can oxidize methane.

Immunochemical investigations of foot-and-mouth disease (FMD). A new component has been found and generally characterized that is produced in tissue cultures or animals infected with FMD virus. This material is distinct from the recognized virus particles and their protein subunits. It has been tentatively termed the "virus infection-associated" (VIA) antigen, because it does not appear to be a constituent of the virus, but is produced as a consequence of the infectious process. The VIA antigen occurs only in FMD infection, but it appears to be immunologically the same regardless of the serological type of FMD virus that induced its formation. For this reason, it may be responsible for many of the difficulties encountered in the diagnostic typing of this disease agent.

Detection of neutralizing antibodies against avian infectious bronchitis. The use of cell cultures for the detection of avian infectious bronchitis neutralizing antibodies was evaluated and shown to be practicable. The test is based on the reduction of virus plaques produced in cell cultures. This test can be completed in two days rather than five to seven days as is the case when the embryonating hen's egg is used. It is also a much more exacting test quantitatively and is free of non-specific inhibiting factors associated with the standard test using embryonating egg assays. With this test 4 cell culture plates will give a more accurate determination of neutralizing antibodies than would 25 embryonating eggs in the standard system.

Viable, avirulent leptospiral immunogen protects against leptospirosis carrier-state. Studies on experimental leptospirosis in hamsters and swine indicate that a newly developed antigen, viz., a selected viable, yet avirulent strain will protect against death, clinical signs of disease and renal infection. Customary bacterins (killed cultures) currently available usually protect against death and clinical signs of disease but permit the establishment of a renal carrier state.

The use of embryonating chicken eggs as the recipient and the donor host in bluetongue transmission studies with Culicoides variipennis. Culicoid flies were infected (100%) by intrathoracic inoculation with egg-adapted bluetongue virus. These infected insects, following 5 days' incubation, were able to transmit bluetongue virus 100% of the time when given a definite blood meal on embryonating chicken eggs. Also, C. variipennis has been infected when given a blood meal on an embryonating chicken egg inoculated with egg-adapted bluetongue virus. This shows the feasibility of using embryonating chicken eggs in bluetongue virus transmission and isolation experiments in the laboratory. Embryonating chicken eggs have the advantage in the laboratory of numbers, economy, and convenience.

Method for controlling anaplasmosis. Under conditions such as those in the hill country of Texas (and areas of the same characteristics) anaplasmosis can be controlled and eliminated from a herd of cattle by the simple procedure of test and separation, together with routine practice of antisepsis. The procedure requires that negative offspring of infected cattle be held in separate pastures from infected cattle. Without accelerated sale or slaughter it has been shown that a totally infected herd can be replaced with negative cattle.

Electron micrographs of crystalline foot-and-mouth disease virus. A significant step has been made in the study of foot-and-mouth disease virus in tissue culture. With the electron microscope, virus particles were seen in very regular crystalline patterns in pig kidney cells. These virus crystals appeared within five hours after the cells were infected. The micrographs represent the first time that foot-and-mouth disease virus has been photographed in a host cell and provides a valuable insight into the mechanism of virus reproduction.

New procedures developed for sampling microbial penetration through the outer structures of the egg. The most common cause of avian salmonellosis in the United States is Salmonella typhimurium, a microscopic bacterium capable of penetrating through the egg shell. Methods have recently been developed which permit the detailed study of the penetration pattern of S. typhimurium through the egg shell only and each of the two thin membranes below the shell surface. These procedures permit the study of eggs in their natural state and are providing important information on the influence of both physical and chemical treatments on bacterial penetration of both hatching and market eggs.

Cross-immunity demonstrated in bovine cooperiasis. Varying degrees of cross-immunity were demonstrated among three closely related intestinal worm parasites (Cooperia punctata, C. pectinata, and C. oncophora) in cattle. These results have important application to the entire problem of parasite control by immunization.

Swine whipworm eggs survive 118 months' exposure on soil at Beltsville, Maryland. Whipworm eggs deposited on the surface of sandy loam soil and buried four and eight inches, respectively, in the vicinity of Beltsville, Maryland, in June and December of 1956 were still infective on April 15, 1966. This observation indicates that the eradication of this parasite from contaminated pastures would be most difficult.

Mature intestinal nematode parasites needed to protect lambs against reinfection. Infection with the intestinal nematode parasite, Trichostrongylus colubriformis, resulting from single doses of infective larvae, failed to provide immunity to reinfection with the same species if the worms were removed with drugs while they were still immature. This indicates that the young worms had not been in contact with the host's tissues long enough to permit the development of an immune reaction against them.

Colostrum-deprived lambs susceptible to the effects of parasitism. Lambs raised entirely parasite-free were clinically affected to a greater degree than were lambs raised helminth-free, except for intestinal threadworms on exposure to comparable infections with sheep stomach worms. Their hematocrits were lower and their weight gains were less, but differences in their worm-burdens as determined at necropsy were not significant.

Comparative tests differentiate between bovine nocardiosis and tuberculosis. A culture filtrate antigen of Nocardia asteroides was compared with mycobacterial antigens in tests on cattle experimentally and naturally infected with N. asteroides and a variety of Mycobacterium species. The results of comparative battery tests for complement-fixing antibodies and cutaneous hypersensitivity clearly distinguished between nocardial and mycobacterial infections.

Transmission of bluetongue virus from vaccinated sheep to susceptible sheep with the vector, Culicoides variipennis. Culicoid flies were infected with bluetongue virus after giving them a blood meal on a bluetongue vaccinated (egg adapted) sheep. These insects thus infected, following a 14-day incubation period, were able to transmit the disease to susceptible sheep.

Transmission of bluetongue virus (BTV) from sheep to cattle and back to sheep with a vector, Culicoides variipennis. Biological transmissions of bluetongue (BT) have been accomplished from sheep to sheep, sheep to cattle, cattle to cattle, and cattle to sheep with the biting midge, Culicoides variipennis. The infected cattle developed a minimal clinical response to BT infection and, therefore, can serve as inapparent carriers of the disease. This information is of great importance to understanding the natural dissemination of the disease in the field from which control measures have to be based.

Effect of Ethoxyquin (Santoquin) on the development of the free-living stages of some nematode parasites of cattle. The antioxidant used in commercial animal feeds, 6-Ethoxy-1,2-dihydro-2,2,4-trimethylquinoline (EMQ), had an inhibitory effect on development of nematode larvae. Increasingly fewer larvae were recovered from feces-vermiculite cultures as the quantity of EMQ fed to infected calves, or mixed with feces from infected calves was increased from the amount commonly used as a feed preservative (1/4 lb/ton) up to 100 times (100x) that amount.

Technique developed for obtaining samples of duodenal fluid from calves. A technique has been developed for opening a fistula into the duodenum for maintenance of a permanent cannula by which continuous samples of the duodenum may be obtained. Such a technique may facilitate ecological studies of the microenvironment of internal parasites as well as studies concerning nutrition and immune reactions of parasites.

Parasite interaction affects specific parasite population. Artificial infection of pigs with the threadworm of swine, Strongyloides ransomi affects the population of other parasites already present in the animal. It has been shown that animals infected with Strongyloides ransomi have half as many or less Ascaris suum. These findings open new fields of investigations in parasite interactions in the swine host.

The effect of vitamin E deficiency on avian encephalomyelitis. Recent outbreaks of avian encephalomyelitis have brought forth the question of coexisting vitamin E deficiency.

Investigations revealed that vitamin E deficiency did not enhance the development of avian encephalomyelitis. In preliminary studies on vitamin E deficiency in chickens, the lesions of exudative diathesis, myodystrophy, and encephalomalacia were produced by varying the amounts of dietary lipid. The common denominator of all three syndromes was damage to blood vessels. The first lesions seen in myodystrophy in chickens was mitochondrial degeneration. This excluded lyosome rupture as the cause of muscle damage as had been previously reported.

As a step toward implementation of the recommendations for a National Program of Research for Agriculture made jointly by the Association of State Universities and Land Grant Colleges and the USDA, a section has been added to each of the Areas in this report. It comprises a list of the related publications of the State Agricultural Experiment Stations in addition to those heretofore reported covering the results of USDA and cooperative research. In future years, it is anticipated that information will be available to permit reporting of achievements resulting from State research in a format comparable to the present reporting of the USDA and cooperative research.

EXAMPLES OF RECENT ACCOMPLISHMENTS OF THE STATE AGRICULTURAL EXPERIMENT STATIONS

Milk-borne immunity found against swine roundworms. It is commonly known that temporary resistance to many microbial diseases in animals is passed from the mother to her offspring by colostrum milk. Disease control procedures in the newborn include this principle as one of the primary methods in preventing losses. There is little information, however, on whether this protective process also occurs in parasitic diseases. Nebraska workers now have found that sows do pass substances in their milk which help protect baby pigs from ascarid worm infection. Baby pigs not immunized by this method had nearly ten times the number of worms as those immunized. The discovery of this principle in swine roundworm infection adds further encouragement of efforts being made to develop protective vaccines against animal parasites.

Immunization against radiation hazards. Cornell scientists have discovered a new principle that has a potential for reducing the damaging effects which radioactive substances have on man and animals. The principle involves immunization against some harmful effects of radiation in much the same way that a person is inoculated against disease. Cell damage from ammonia released in the intestine as a result of radiation has been found to be a significant factor contributing to death of the animal. Toxic accumulation of ammonia can be prevented by immunization against urease, a body enzyme which breaks urea down to ammonia. Although cell injury also results directly from radiation, the reduction in ammonia gives the cell a better chance to overcome the damaging effects of radioactivity. Cornell scientists foresee future applications of this discovery in protecting cancer patients undergoing exposure to high doses of radioactivity as well as scientists working in areas of potential radiation hazard.

New procedure for controlling egg-borne Salmonellosis. The occurrence of Salmonella in chickens sometimes results in bacterial contaminated eggs, particularly in those eggs in which cracks or checks allow the organism to penetrate the shell barrier. Uncooked egg products contaminated in this manner may cause illness when consumed. The South Carolina station has found a practical method for reducing this potential Salmonella infection. Eggs are washed in water raised to a temperature and for a time period high enough to kill all bacteria but without causing heat coagulation of the egg protein. This process has been found to render heavily contaminated eggs safe for consumption.

A new cause of swine infertility discovered. Pennsylvania scientists have uncovered a new cause for reproductive losses in swine. The agent has been found to be a virus which may lead to herd outbreaks of stillbirths, abortions, and infertility. While bacterial and nutritional diseases have

been known to cause similar problems, no virus other than hog cholera has been known to cause widespread reproductive losses in swine. This finding will greatly aid in diagnosing outbreaks of reproductive failure in swine and may eventually lead to methods for effective prevention.

Vaccines being developed for the prevention of transmissible gastroenteritis (TGE) in swine. Scientists at Indiana and Illinois Experiment Stations are evaluating vaccines for the prevention of TGE. It is estimated that 20-30 percent of all young pigs die before they reach 8 weeks of age. Infectious diseases, including TGE, are responsible for a significant part of these losses.

Protection from TGE is transmitted via the sow's milk and a continuous supply of "immune" milk is necessary in order to provide protection. The virus in the vaccines is grown in cell cultures and inactivated. The vaccine is administered in two doses, the last dose being given at least 30 days prior to farrowing. Present vaccines show promise of affording effective protection under field conditions.

Possible insect reservoir discovered for leukosis. Scientists at the Georgia station have found that a scavenger beetle which often abounds in poultry litter, is capable of harboring the leukosis virus of chickens and may pass the infection on to susceptible birds. While other means of spread have been known for this widespread, cancerous condition of poultry, scientists have felt that some unknown local factor also may initiate the disease in a flock. This research now suggests that the scavenger beetle may be a source of leukosis infection in a particular locality and the cause of flock outbreaks.

A new test for the diagnosis of equine infectious anemia. Research workers at the Texas station have developed a reliable laboratory test for diagnosing equine infectious anemia. Before the development of the test, inoculation of susceptible horses was the only reliable method of diagnosing the disease. A positive test depends on the presence of an abnormal serum protein. The diagnostic reagent is now being prepared by growing the virus in cell cultures and injecting the material in sheep. Attempts are continuing to improve the test.

AREA NO. 1 - INFECTIOUS AND NONINFECTIOUS DISEASES OF CATTLE

Problem. Losses from infectious and noninfectious diseases of cattle, other than those due to parasites, are estimated at approximately $600 million annually. These losses materially increase costs of production and conversely decrease profits. In turn, they contribute to the cost of every purchase of meat, milk, and other cattle products to the consumer. Some of these diseases are transmissible to man. Determination and definition of the causes of cattle diseases, explorations for efficient methods of diagnosis, prevention, control, and when feasible, eradication, are the purposes of the research program.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving biochemists, microbiologists, pathologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of infectious and noninfectious diseases of cattle. Research is being conducted on the diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 31.3 scientific man-years. This effort is divided among sub-headings as follows:

Vibriosis of Cattle 2.0 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the New York State Veterinary College at Ithaca.

Tuberculosis of Cattle 2.0 at the National Animal Disease Laboratory, Ames, Iowa, and through a contract with the Michigan State University at East Lansing.

Mucosal-Respiratory Disease-Complex 3.0 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreement with Iowa State University, Ames.

Mastitis of Cattle 3.5 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the University of California, Davis.

Epizootic Bovine Abortion 0.5 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the University of California, Davis.

Foot Rot (Infectious Pododermatitis) of Cattle 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Etiological, Cytological and Histochemical Studies of Pulmonary Adenomatosis in Cattle 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Immunization against Bovine Leptospirosis 1.5 at the National Animal Disease Laboratory, Ames, Iowa.

Chemotherapy in Leptospirosis 1.5 at the National Animal Disease Laboratory, Ames, Iowa.

Enteritis of Young Calves 0.5 at the National Animal Disease Laboratory, Ames, Iowa, and under a contract with the University of Idaho, Moscow.

Bovine Lymphosarcoma 3.3 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the University of Nebraska, Lincoln, Nebraska and Cornell University, Ithaca, New York.

Respiratory Disease of Cattle (Shipping Fever) 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Brucellosis of Cattle 2.5 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the University of Minnesota, the University of Wisconsin, and with the Ohio Agricultural Experiment Station. A project on the immunizing effect of Brucella cell wall is in progress at the Hebrew University, Jerusalem, Israel, under a PL 480 Grant of funds equivalent to $31,950.00 over a 3-year period.

Paratuberculosis of Cattle (Johne's Disease) 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Keratitis (Pink-eye) 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Poisoning by Plants 1.0 at the Logan, Utah, Field Station, through formal cooperation with the Utah Agricultural Experiment Station. A PL 480 grant supports research at the Instituto Biologico, Sao Paulo, Brazil, on The Study of Plants of the State of Sao Paulo Poisonous to Domestic Animals.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 143.5 scientific man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Vibriosis.

The New York State Veterinary College, Cornell University at Ithaca, under a cooperative agreement with the USDA, reported that minor modifications have been made in the preparation of the fluorescent conjugate and in the manner of processing preputial samples, in order to increase the efficiency and sensitivity of the fluorescent antibody test for detecting V. fetus carrier bulls. A comparison of the sensitivity of fluorescent antibody (FA) and cultural techniques is being made on preputial samples.

Distribution of V. fetus and V. bubulus in the Preputial Cavity of the Bull. Work is being completed on a project designed to determine the distribution of V. fetus and V. bubulus in the preputial cavity and to ascertain whether the presence of V. fetus stimulates a local inflammatory and immune response. Results of this study indicate that the highest concentration of the organism is on the dorsal surface of the penis and in the posterior portion of the preputial cavity, where its preferential site appears to be within the epithelial crypts. V. fetus does not penetrate the epithelium nor does it induce an observable inflammatory response. V. bubulus is localized largely in the anterior portion of the preputial cavity.

The Chemical Composition and Antigenic Structure of the Heat-labile Surface Antigen of V. fetus. Experimental evidence indicates that this antigen is not an acidic polysaccharide, as are the Vi antigens of Salmonella and the K antigens of the E. coli, and that it forms an incomplete layer over the heat-stable cell wall antigens.

Treatment of V. fetus Carrier Bulls. Twenty-seven V. fetus carrier bulls in the local AI unit were treated with hydrogen peroxide and furaltodone hydrochloride in an effort to abolish the carrier state. Despite the implementation of procedures designed to avoid the possibility of reinfection, 18 of 27 bulls remained carriers. Eight of the carrier bulls were retreated by a modified procedure in which the concentration of furaltodone was raised and the solution was massaged in the preputial cavity for a longer period. Five of the 8 bulls were cured by this treatment. (Ithaca, New York) (ADP al-9(Rev.))

The National Animal Disease Laboratory (NADL), Ames, Iowa, reports that twenty-four sexually mature virgin heifers were treated during estrus as follows. Six were inoculated intracervically with viable V. fetus followed immediately by artificial insemination. Fifteen others were divided into 3 equal groups which were given in utero inoculations of V. fetus cell-fraction materials (intracellular material, cell wall, and endotoxin material), followed immediately by artificial insemination. Three control heifers were given 0.85% NaCl solution in utero and 2 were artificially inseminated. The heifers were necropsied 7 to 93 days after exposure. The genital tracts were examined bacteriologically, histopathologically, and grossly for signs of pregnancy.

The 6 heifers receiving viable V. fetus became infected in the vagina, and 5 of them became infected in the uterus. The lesion was classified as a subacute diffuse mucopurulent endometritis, characterized by the accumulation of exudate in the lumen of the uterine glands and a marked periglandular lymphocytic infiltration.

The genital tracts of the 15 heifers receiving cell-fraction materials were histologically normal at necropsy. Only the 2 inseminated control heifers became pregnant as far as could be determined.

A study of the antigenic components from V. fetus has been undertaken and partially completed. Two antigens from the protoplasm, common to several strains of V. fetus, have been isolated and chemically characterized. A third antigen from the cell wall of several strains has been extracted, purified, and partially characterized by chemical and physical-chemical techniques. This antigen, an endotoxin, has also been assayed for its toxicity in mice. Further work is now in progress to characterize the lipid component thought to be responsible for toxicity and perhaps the pathogenesis of this disease. (Ames, Iowa) (ADP al-40)

B. Tuberculosis .

Research studies were continued at the National Animal Disease Laboratory, Ames, Iowa, as follows:

A review of methods applied to studying the antibody response of infected animals (and man) has shown that no reported serologic method has proved highly specific and sensitive in diagnosing tuberculosis. A possible exception is the direct agglutination test with poultry serum.

Changes in the relative concentration of chemical components found in serum often have diagnostic significance. However, when serum glycoproteins were studied in tuberculous cattle, only experimentally infected cattle showed significant changes in the concentration of glycoprotein. These changes were of short duration and did not persist in cattle known to be infected with Mycobacterium bovis.

The hypersensitivity to tuberculin of cattle infected with M. bovis continues to be the basis for detecting cattle infected with M. bovis. The test used in the control program consists of inoculating tuberculin in the caudal fold. It is known, however, that the skin is more sensitive at other portions of the body, in particular the cervical region. It is also known that cattle infected with Mycobacterium paratuberculosis (Johne's bacillus) often show positive reactions to intradermal inoculations of tuberculin. These facts were used in a study to determine if less concentrated tuberculin inoculated in the sensitive cervical region could more accurately diagnose tuberculosis than is accomplished with the conventional caudal fold test. In the portion of the study that has been completed, it was found that simultaneous inoculations of 2 tuberculin concentrations did not significantly affect the size of reactions to either concentration when used in cervical tests. These tests, conducted with cattle naturally infected with M. paratuberculosis can be used as a base for continuing investigations of tuberculin sensitivity in tuberculin-sensitive cattle regardless of the cause of sensitization.

In a herd of 156 cattle, blood samples were collected for conducting the complement-fixation test for tuberculosis, both before and after (4 weeks) testing with intradermal johnin and tuberculin tests. The injection of tuberculin and johnin did not cause the development of positive complement-fixation titers to bovine tuberculosis or change titers already present.
(Ames, Iowa) (ADP al-13(Rev.))

Research was continued at the Michigan State University, East Lansing, under contract with the USDA.

Contract 12-14-100-7164(45). The final report on this contract was received during fiscal year 1966.

Section I - Studies were made of the relative virulence of 12 cultures of mycobacteria in various animal species. The cultures included 2 M. bovis, 2 M. avium, 7 Group III mycobacteria and one saprophyte culture. A method of evaluating the pathogenicity of mycobacterial cultures by injecting intradermally at seven sites 10-fold dilutions of each culture, starting with 0.1 ml (1 mg wet weight of cells) was tested and gave a usable answer. To evaluate the results on each animal for 8 weeks, an infectivity index was developed that rates the average size of the reation and the highest dilution in which a reaction develops at the inoculation sites.

Although variations in pathogenicity were observed, the relative pathogenicity as shown by the pathogenicity index, was comparable for guinea pigs and calves. Thus, the guinea pig can be used for measuring pathogenicity of mycobacteria for calves. The gross and histopathologic examinations were in keeping with the pathogenicity indices.

Much higher indices were obtained for swine than for guinea pigs, except for M. bovis strains, where the indices were approximately the same.

In general, the pathogenicity indices for rabbits were much higher than for calves, swine and guinea pigs.

All mycobacteria produced some degree of reaction at one or more inoculation sites on chickens. M. avium produced a relatively large area of reaction with an ulcer that continued for eight weeks. M. bovis produced a slightly smaller area of reaction with an ulcer, but recovery occurred.

The Group III mycobacteria produced a much smaller area of reaction without ulcers and rapid disappearance of the reaction. Saprophytes produced only a slight reaction that disappeared in three weeks. The intradermal injection of chickens for typing mycobacteria has possibilities for routine use.

Section II - When Group III mycobacteria of both high and low virulence were injected into calves and adult cattle, both were infected. The

highly virulent culture generally produced generalized disease in the calves but only primary complex disease in the adults. However, it should be noted that gross lesions were produced in both calves and adults. These data definitely demonstrate that Group III mycobacteria can produce disease in adult cattle and cause hypersensitivity to mammalian tuberculin.

Section III - As would be expected, a tuberculin consisting of unheated filtrate of M. bovis produced reactions on guinea pigs sensitized with M. avium, pathogenic Group III mycobacteria of bovine, swine, and human origin. Reactions were produced on pigs sensitized with Group III nonpathogenic strains. Tuberculins made from some of these organisms also had broad sensitivity spectrums. Leakage material was extracted from the same organisms by suspending in buffered normal saline and freezing. The resulting filtrates were used as sensitins. These products were far more specific than were the tuberculins. When these leakage materials were examined in an infra-red spectrophotometer, the only substances detected were nucleic acids.

Section IV - Antibody production was markedly altered, qualitatively and quantitatively by the source, preparation and combinations of antigens; route(s) the antigen was administered; the schedule of inoculations; and many other variables. The method of choice must be determined empirically for each kind of antigen-antibody combination and the system by which it is detected.

Serums from rabbits inoculated with Beta propriolactone inactivated cells of a selected group of mycobacteria were tested with unheated filtrate or freeze-thaw extract using the single diffusion gel slide test and the double diffusion gel slide test using homologous and herterologous systems. The results of this study indicate clearly that further studies should be made to identify and separate those fractions which will identify the isolants.

Other findings under this contract were presented in the annual report for 1965. (Michigan) (ADP a1-13(Rev.))

During the fiscal year investigations were initiated at Michigan State University, to develop in vitro cytotoxic procedures for the study of tuberculo-sensitivity. (East Lansing, Michigan) (ADP a1-38(C))

C. Mucosal-Respiratory Disease-Complex of Cattle .

Research workers at the National Animal Disease Laboratory, Ames, Iowa, reported as follows:

Serums from calves inoculated with a cytopathogenic bovine viral diarrhea (BVD) virus, a noncytopathogenic BVD virus, or with a soluble antigen (SA) extracted from a BVD virus-infected cell culture were fractionated into heavy (19S) and light (7S) components of gamma-globulins by means of ultra-centrifugation in a sucrose gradient. These antiserum fractions were tested for precipitating antibodies, for fluorescing antibodies, and for their capacities to neutralize BVD virus in cell cultures. Most 19S fractions elicited lines of precipitation with a SA of BVD virus, but only the 7S fraction produced from injections of SA into a calf precipitated, and only weakly, with the BVD-SA in agar-gel double diffusion plates. All fractions, except a 19S, combined with viral antigen produced in swine kidney cell-line (PK-15) cultures in an indirect fluorescent antibody test.

The 19S fraction predominated in the bovine serums studied and persisted for as long as 20 weeks. Restimulation of animals with antigen did not produce a secondary effect and the "early 19S", "late-7S" responses usually present in most immunologic systems were not present in this immunologic system. The type of antibody produced in this bovine system and the response of bovine species to this viral agent is different from those reported for many other viruses infecting other hosts. These results confirm and re-affirm the concepts that animal immunologic systems are extremely heterogeneous. (Ames, Iowa) (ADP a1-14(C)(Rev.))

Iowa State University under cooperative agreement with the USDA has shown by neutralization kinetic studies, single-step growth curve studies, and thermostability studies that a previously isolated strain of IBR, which had a tendency to generalize in the bovine, differed markedly from nonvirulent strains. The virulent strain killed cells in culture sooner, released fewer virus particles per cell, and was released from cells more rapidly. By reciprocal cross neutralization kinetic studies, the virulent strain was shown to fall into a unique group. Similarly, the virulent strain was more thermolabile than the nonvirulent strains. These results suggest that virulence is a strain characteristic in that fine antigenic changes (protein changes) are related to virulence. They theorize that these fine changes in the protein coat can more effectively inhibit normal cellular metabolic processes, and possibly are responsible for a wider cell trophism that would imply a wider organ involvement during an active infection.

A plaque characterization method has been devised in which plaque size and morphology can be used as a method of isolation and identification. It has been demonstrated that this method can be used in primary isolation and will detect combined infections, as well as titrate mixed dual and triple modified live vaccines.

A series of fetal inoculations with BVD virus produced abortions in 5 of the animals, 1 anomaly, and 3 normal calves. These normal calves were born with antibodies, and on challenge responded as immune individuals.
(Ames, Iowa) (ADP al-14(C)(Rev.))

D. Mastitis of Cattle .

The following work has been reported from the National Animal Disease Laboratory, Ames, Iowa.

Differential pressures across the bovine teat canal and the sequence of events during milk removal by hand milking, machine milking, and calf suckling were studied with two cows and their 10- and 15-day-old calves. The teat sinus was cannulated and a polyethylene catheter passed into the teat sinus. During suckling experiments a second catheter was anchored to the teat and udder skin. A pulsator rate of 48 per minute and a milking vacuum of 12.5 in. Hg were used during mechanical milking. The average frequency of hand milking and calf suckling was 67 and 117 cycles per minute, respectively. Average differential pressures across the teat canal were 310 mm. Hg for hand milking, 352 mm. Hg for mechanical milking, and 535 mm. Hg for calf suckling. The udder appeared to be evacuated most rapidly during calf suckling, which was attributed to the rapid suckling cycle and the high differential pressure across the teat canal.

The difference in susceptibility of streptococci of Lancefield group A (Streptococcus pyogenes) and Lancefield group B (Streptococcus agalactiae) to the antimicrobial system in milk (lactoperoxidase and thiocyanate) was studied. It was found that S. pyogenes is obligated to use a fermentative pathway for obtaining growth energy, whereas, S. agalactiae can use both a fermentative and an oxidative pathway. One of the critical enzymes of the fermentative pathway in S. pyogenes, glyceraldehyde phosphate dehydrogenase, was shown to be inhibited by the lactoperoxidase system. This enzyme is a likely site of action of the inhibitor. The oxidative pathway possessed by S. agalactiae allows the energy metabolism to proceed even though the fermentative pathway is blocked by the lactoperoxidase system. This may explain why S. pyogenes is prevented from growing in milk whereas S. agalactiae is only delayed but not completely inhibited in its growth.

As a means of differentiating strains of Staphylococcus epidermidis isolated from bovine udder infections, pigment production of the cultures was

studied. The cultures were grown on a medium containing cream at 30°C. for 2 days and the color characterized numerically by determining the dominant wavelength, purity and brightness using a colorimeter with a reflectance attachment. This method generally agreed with the visual grading of the colors and would be of value as an objective method for studying quantitative differences in pigment production. It was concluded that the method is better for determining differences of pigment production within strains of S. epidermidis after they are first differentiated by the type of spectral absorption curve produced by the pigments extracted from the cells with methanol (See 1965 report).

Tergitol-7 medium with triphenyltetrazolium chloride (TTC) has been used for distinguishing the various coliform organisms. While using the medium, it was observed that the Escherichia coli organisms produced more than one type of colony. A study of 251 E. coli cultures isolated from 92 animals showed that four types of colonies developed. Three types, rough, intermediate, and mucoid, were yellow to amber and produced slight yellow zones in the medium. The fourth, a tetrazolium reducing type, was red with blue zones in the medium. The rough and intermediate type cultures appeared to be more closely related biochemically than either type to the mucoid or tetrazolium-reducing types. Serological O-group typing of 72 cultures by Pennsylvania State University showed that only one O group was shared by the rough and intermediate type strains and both cultures were isolated from udder infections in different cows. The use of Tergitol-7 medium with TTC might be of value for screening purposes in epizootiological studies of E. coli infections. (Ames, Iowa) (ADP a1-15)

The University of California, Davis, under a cooperative agreement with the USDA reported as follows: When the mammary gland comes into full milk flow, the pre-existing cellular reaction and inhibitory serum factors in colostrum are suddenly lowered by dilution with milk. Acute mastitis may follow as minimal numbers of pathogens, either pre-existing in the gland or suddenly entering the gland, begin to multiply rapidly. It was conjectured that if a modest inflammatory response existed at freshening, acute attacks of mastitis might possibly not occur. The level of inflammatory reaction that would result from injection of 1.0 million heat-killed coliform organisms into lactating glands was investigated. Studies showed that a mild inflammation could be induced by such means. Heat-killed Aerobacter aerogenes suspended in saline and sealed in ampules retained inflammation-inducing properties for at least 7 months when held in the refrigerator.

To determine the effect of induced leukopenia on experimental A. aerogenes mastitis, a trial employed a sublethal anaphylactic reaction to egg albumin drip over a 10-hour period to induce neutropenia during the initial phase

of growth of A. aerogenes in a lactating quarter.

The trial was successful in that neutropenia was produced within 10 minutes of starting the egg albumin drip and was maintained for 45 hours. A. aerogenes inoculum in the quarter declined in numbers during the first 3½ hours but then multiplied rapidly through the 16th hour. Swelling of the gland, expected in 4 to 5 hours in a normal inoculated quarter, was delayed until the 10½ hours postinoculation of A. aerogenes. The clinical disease resulting was more protracted than in a normal cow. An initial leukocytosis of 10 million neutrophils/ml. at the 16th hour dropped to less than 1 million/ml., between the 17th and 20th hours, as blood serum factors entered the milk (udder fluid serous and clotted). At the same time A. aerogenes numbers also became drastically reduced.

A very significant event in this trial was the spontaneous development of acute mastitis in an opposite quarter (RR) which had a natural infection from coagulase-negative staphylococci. The number of these bacteria was 300/ml. and cell count 380,000 at start of the drip. By the 24th hour of leukopenia, swelling was noted in the RR. The staphylococcal count was 500,000/ml. and neutrophil leukocytes 560,000/ml. The swelling became very severe and by the 45th hour, the staphylococcal count was 68 million/ml. and neutrophil count was 98 million/ml. This was a vivid demonstration of a severe mastitis produced by a relatively harmless organism when general body defenses were brought to a low level by another cause.

A series of experiments was carried out in attempts to characterize the bactericidal system in cow blood for Gram-negative bacteria that can enter the milk in acute mastitis. It was found that the bactericidal activity of milk did not correlate completely with the degree of udder inflammation; that the bactericidal activity of mastitic milk could be absorbed out by dead bacteria; that bactericidal activity was not associated with lactenin; and that the bactericidal activity of the serum was probably associated with the gamma 1-macroglobulin fraction. Colostrum had very little bactericidal activity. (Davis, California) (ADP a1-15)

E. Epizootic Bovine Abortion.

The National Animal Disease Laboratory, Ames, Iowa on the psittacosis-group agents in birds and domestic animals reported as follows:

In the study of relationships among psittacosis-LGV-trachoma agents found in wild birds and in domestic mammals, 4 strains were titrated in 8 species of animals to compare their present infectivity and lethality for each host. The strains were isolated from (1) a turkey with acute ornithosis, (2) a

pigeon without gross lesions, (3) an aborted ovine fetus, and (4) a lamb with polyarthritis. The host species were mice, guinea pigs, pigeons, sparrows, parakeets, turkeys, lambs, chicken embryos, and turkey embryos.

The titration experiments revealed that the 4 strains had sufficiently distinct pathogenicity spectrums that they could be differentiated on the basis of their effect on 3 of 8 host species: mice, guinea pigs (both inoculated intraperitoneally), and pigeons (inoculated intracerebrally). Small numbers of the virulent turkey ornithosis agent were lethal for mice and guinea pigs, but high concentrations failed to cause lesions in pigeons. The pigeon ornithosis agent produced lesions in mice and pigeons but failed to affect guinea pigs. The sheep abortion agent produced mild lesions in all 3 species when high concentrations of it were inoculated. In contrast, the sheep polyarthritis agent in high concentrations failed to affect mice and pigeons but caused lesions of severe ornithosis in guinea pigs.

Observations of potential significance to the understanding of natural inter-species transfer were that the pigeon ornithosis and sheep polyarthritis agents produced severe aerosacculitis in intraperitoneally inoculated turkeys. The polyarthritis agent not only produced crippling inflammation of the leg joints and tendons of lambs, but also severely affected the hocks of turkeys. The sheep abortion strain was infectious for pigeons and lethal for sparrows, suggesting a possible role of these wild birds in the interflock transmission of that agent.

Studies on the pathogenicity of avian and mammalian psittacosis agents for laboratory animals were extended to include two strains isolated from cases of sporadic bovine encephalomyelitis (SBE) and two strains isolated from cases of epizootic bovine abortion (EBA). The significant findings were: (1) in terms of pathogenicity for laboratory animals, the California strain causing EBA was identical to the previously tested California pigeon psittacosis strain. This meant that this strain produced severe systemic infection in mice, pigeons, sparrows, parakeets, and turkeys which was indistinguishable from psittacosis. This strain had been reported as causing abortion in cattle and fatal psittacosis in a husbandryman in California. (2) The pathogenicity spectrum of the SBE agent was in most respects identical to a previously tested ovine polyarthritis strain, except that it did not cause arthritis in lambs.

This work indicates that the pathogenicity tests are useful as markers for identifying strains in terms of pathotype, thereby assisting in determining the original source of the disease agent.

The University of California, Davis, under a cooperative agreement with the USDA reported that vaccination with either inactivated or viable attenuated (or partially attenuated) EBA virus, or both, provides little protection against challenge exposure with virulent EBA virus given under experimental conditions. The efficacy of these vaccines, when given in early gestation, will be investigated on a laboratory scale and field trial basis. Cattle exposed to virulent EBA virus at the time of breeding displayed an enhanced resistance to challenge virus given subsequently. This observation will be studied further as a possible method of immunizing against EBA.

Liquamycin, a tetracycline compound, showed little or no prophylactic or therapeutic activity under experimental conditions in cattle.. The possibility will be studied that tetracycline compounds, administered to cattle in the form of feed additives, will prove effective.

An agent belonging to the psittacosis-lymphogranuloma-venereum (PLV) group isolated from Ixodes pacificus ticks removed from a dog produced abortion in the only cow inoculated. A second isolate, recovered from Dermacentor occidentalis ticks, has failed thus far to produce abortion in a cow. These studies will be continued to determine the identity of these isolates and the possible epizootiologic significance in EBA of the species of tick from which they were isolated.

The first step toward developing a serum neutralization (SN) test for EBA has been accomplished in that the EBA agent has been propagated in cell culture, accompanied by the production of cytopathic changes in the cell monolayer.

(Davis, California) (ADP al-21 (Rev.))

F. Foot Rot (Infectious Pododermatitis).

The National Animal Disease Laboratory, Ames, Iowa, reported that warts produced in the feet of 6 cattle under simulated barnyard conditions had almost disappeared 8 weeks after inoculation. Although papillomas were produced along the coronary bands, in the bulbar regions of the feet, and in the interdigital tissues, the typical ulcerative lesions of foot rot did not appear.

A second group of 6 cattle were placed in pens containing a muck composed of mud or peat moss, which resembled muddy barnyard conditions, similar to that used in the wart virus study. The feet of the cattle were inoculated in the skin of the coronary bands, in the bulbar regions of the feet, and in the interdigital tissues with a culture of Dermatophilus congolensis. No lesions developed in the feet during the next 6 weeks. At the end of

the 6-week period, the same areas of the feet previously inoculated were again exposed with D. congolensis. No lesions appeared in the feet but the animals developed antibodies against D. congolensis.

A third group of 6 cattle were placed in a mud or peat moss muck similar to that used in the previous 2 experiments and were inoculated in the coronary bands, in the bulbar regions of the feet, and in the interdigital tissues with the New Jersey strain of vesicular stomatitis virus. Typical lesions of vesicular stomatitis developed and healed. The chronic eroding lesions of common foot rot did not appear.

These experiments have shown that muck composed of peat moss or dirt mixed with feces and urine simulating muddy barnyard conditions will not produce foot rot. The rapidity with which healing occurred in cattle affected with wart virus and vesicular stomatitis virus suggested that muck may actually have a beneficial effect and promote healing.

(Ames, Iowa) (ADP a1-22)

G. Bovine Pulmonary Adenomatosis.

The National Animal Disease Laboratory reported as follows:

Experimental inhalation of nitrogen dioxide by cattle resulted in methemoglobinemia, severe dyspnea and death. Pulmonary lesions consisted of hyperemia, edema, hemorrhage, fibrin deposition, hypertrophy and hyperplasia of bronchial and bronchiolar epithelium, alveolar epithelial proliferation, chronic proliferative bronchiolitis, alveolar and interstitial emphysema and infarction. Emphysema was also prominent in the mediastinum as well as the mediastinal and bronchial lymph nodes. There was diffuse coagulative necrosis of proximal convoluted tubules. In addition to multiple pulmonary and renal thrombi, edema and hyalinization of the walls of several vessels were present.

In studies of rumen insufflation by nitrogen dioxide in 4 cattle the only fatalities resulted from excessive methemoglobinemia. Minimal pulmonary lesions occurred. These were mild bronchial and bronchiolar epithelial hyperplasia, hyperemia, edema, atelectasis and emphysema. Coagulative necrosis of the proximal convoluted tubules was prominent. Rumen necrosis was severe in the area of gas introduction. There was general passive hyperemia. No proliferation of alveolar epithelial cells occurred as is characteristic of adenomatosis in cattle. Thus rumen origin nitrogen dioxide probably is not involved in bovine pulmonary adenomatosis.

(Ames, Iowa) (ADP a1-24)

H. Leptospirosis.

At the National Animal Disease Laboratory, Ames, Iowa, attempts were made to reduce the infectiousness of L. pomona while preserving its immunizing activity. It was found that L. pomona was rendered nonreplicating by exposure to dihydrostreptomycin or γ-rays (1×10^5 rads) but that such organisms conferred to hamsters and swine considerable protection against infection with renal trophic strains of L. pomona.

Separate investigations have determined a way to select strains of Leptospira pomona capable of good growth in a nonantigenic, synthetic medium. Such organisms were avirulent but highly immunogenic for hamsters and swine. The development of a viable, avirulent strain for immunization, on the basis of preliminary evidence, offers considerable promise in the control of leptospirosis of domestic animals.

(Ames, Iowa) (ADP al-25)

The National Animal Disease Laboratory, Ames, Iowa reported that although several antibiotics and dyes prevent the multiplication of leptospires in the test tube, only dihydrostreptomycin and perhaps chlortetracycline are effective against leptospires localized in the renal tubules of an infected hamster. Injections of dihydrostreptomycin also cure renal leptospirosis in swine but, unfortunately, the drug is not absorbed when administered in the feed or drinking water.

Chlortetracycline in the feed will cure renal leptospirosis in swine but only if fed at a level which is twice the amount permitted by the present regulations on feed additives.

(Ames, Iowa) (ADP al-26)

I. Enteritis in Young Calves.

The Caldwell Veterinary Research Laboratory, University of Idaho, under a contract with the USDA reported progress in the first phase of studies on resistance to enteritis (scours) among calves. Forty head of beef bred cows and 2 beef bred bulls were selected, purchased and brought to the station in accordance with provisions of the contract. In accumulating the data which furnished the basis for the selection of these animals, a total of 984 serums were separated electrophoretically.

(Caldwell, Idaho) (ADP al-29(C))

J. Bovine Lymphosarcoma.

The National Animal Disease Laboratory, Ames, Iowa, reports that additional field cases of bovine lymphosarcoma have been investigated making a total of 12. Ten of the 12 cases have been confirmed by necropsy while 2 were instances of mistaken diagnosis. In all but one case (LS-11), involving an Angus calf approximately 5-1/2 months old, there have been successful isolations of Trypanosoma theileri. In the last instance, T. theileri was isolated from the dam.

Tissue cultures prepared from spleens or lymph nodes of animals involved in 4 cases -- LS-5, LS-10, LS-11, and LS-12 -- developed resistance to challenge exposure with vesicular stomatitis virus. The agents responsible for inducing resistance to the cultures in 2 cases -- LS-5 and LS-10 -- have been successfully transferred by cell-free medium to additional cultures of bovine embryonic spleen. It appears likely that transfer of resistance by cell-free medium from the 2 remaining cases will be accomplished.
(Ames, Iowa) (ADP a1-30)

The Animal Health Division in cooperation with the Animal Disease and Parasite Research Division is conducting epizootiological studies of bovine lymphosarcoma at the University of Pennsylvania, Philadelphia, Pennsylvania, the South Jersey Medical Research Foundation, Camden, New Jersey, and at the University of Minnesota, Minneapolis, Minnesota. This program is expected to make specimens of more than usual research value available to the National Animal Disease Laboratory, Ames, Iowa, for further detailed study.
(ADP a1-30)

The Agricultural Experiment Station, University of Nebraska, reported on a recently initiated cooperative agreement with the USDA. To date they have been unable to demonstrate T. theileri from the blood of 2 hysterectomy-derived, colostrum-deprived calves given tumorous material from a cow that died of leukemia.

Immuno-diffusion techniques have been attempted to demonstrate antigen-antibody reaction among serums from positive animals and antigen prepared from lymphoma tissue. Further refinement of procedure and antigen must be made as results have thus far been negative.
(Lincoln, Nebraska) (ADP a1-30)

The New York State Veterinary College, Cornell University, Ithaca, submitted the following report on a cooperative agreement with the USDA.

Ten cows with lymphosarcoma have been subjected to intensive studies in an attempt to gain evidence on the cause and development of this disease. Plasma, milk, and tumor tissue have been examined by ultracentrifugation,

immunologic, and electron microscopic methods. Tumor cells have been grown in test tube and bottle cultures, in order to study their growth characteristics, chromosomes, and possible virus infection. Materials from blood, milk, and tumor tissues have been accumulated for long-term research on immunity. Up to the present, no evidence of a bovine leukemia virus has been demonstrated in these studies.

(Ithaca, New York) (ADP al-30)

K. Respiratory Diseases of Cattle.

The National Animal Disease Laboratory, Ames, Iowa, in continuing studies on the physiology of organisms associated with respiratory diseases of cattle reported that chemically defined medium for the growth of P. haemolytica was developed. It consisted of 15 L-amino acids, inorganic salts, citrate, vitamins (nicotinamide, pantothenate, thiamine or its monophosphate) and 1% D-galactose plus 0.1% D-glucose. Aerobic cultures produced populations as high as 2×10^{10} cells per ml., equal to that of complex mediums. A large number of strains of P. haemolytica, from many sources, grew well in the medium, as did strains of P. multocida tested. The medium is being used in studies on the effect of bovine tissue exudates and fluids upon proliferation of Pasteurella sp.

(Ames, Iowa) (ADP al-31)

L. Brucellosis of Cattle.

At the National Animal Disease Laboratory, Ames, Iowa, several solid mediums were compared for their efficacy in isolation of Brucella from infected tissues. Tissues were obtained from guinea pigs infected with Br. abortus, type 1, Br. abortus, type 2, Br. melitensis, type 1, Br. suis, type 1, or Br. suis, type 3. Additional tissues were obtained from cattle infected with Br. abortus, type 1 and from swine infected with Br. suis, type 1 or Br. suis, type 3. The mediums compared were: Tryptose agar, trypticase soy agar, potato infusion agar, Albimi brucella agar, modified tryptose agar, tryptose-serum agar, modified tryptose-serum agar, potato-serum agar and tryptose-serum-antibiotics-dye agar. The first six mediums were also compared for their ability to support the growth of laboratory-adapted Brucella cultures.

There were only slight differences among the mediums in their ability to support the growth of in vitro propagated strains. However, when the mediums were inoculated with Brucella-infected tissues, there were distinct differences in the isolation efficacy.

Serum-enriched mediums were consistently superior to the basal mediums. Tryptose-serum agar containing antibiotics and ethyl violet was nearly as efficient as nonselective mediums for isolating Brucella. The value of more

than one medium for isolation was demonstrated. Mediums for routine use in isolation of Brucella should include a selective medium as well as one of the basal mediums enriched with bovine serum.

(Ames, Iowa) (ADP al-32)

The University of Minnesota, under a cooperative agreement with the USDA, reported that studies during the past year have been concerned with (1) further evaluation of supplemental seroagglutination tests that may improve the efficacy of diagnosis of the disease among cattle subjected to the market cattle testing program (MCT) at the time of slaughter; (evaluation of initial studies indicated that one or more of the supplemental tests may be more efficient in detecting herds with infection than the standard test); (2) further studies and comparisons of problem herds and herds with single reactors and no other evidence of brucellosis; and (3) investigation and epidemiologic studies of a naturally occurring outbreak of brucellosis of sheep accompanied by abortion - the first reported in North America due to Brucella abortus.

(St. Paul, Minnesota) (ADP al-32)

The Ohio Agricultural Experiment Station, Wooster, under a cooperative agreement with the USDA reported on a study to determine the resistance of pregnant cows to Brucella abortus 2308 following vaccination with Brucella abortus, Strain 19 vaccine at 2 or 3 months of age. Of the 10 heifers, vaccinated at 2 months of age, 3 became infected (30%) and 2 aborted. Three of 8 (37.5%) animals vaccinated at 3 months of age became infected, and 2 aborted. Of the 6 pregnant nonvaccinated controls, 4 became infected (66.6%) and aborted. In addition, 1 nonpregnant nonvaccinated heifer was found to be infected at necropsy. A comparison of these results with those previously obtained with calves vaccinated at 4 to 8 months is being made.

(Wooster, Ohio) (ADP al-32)

The University of Wisconsin, Madison, under a cooperative agreement with the USDA reported that in their studies the complement fixation test was the most effective of the supplementary serological tests for the detection of infected animals regardless of the tube agglutination titer. During the year, more than 25,000 serum samples were tested with the CF test.

A systematic study on serological response to Strain 19 vaccinated and nonvaccinated cattle was initiated. Sixty-four female Holstein cattle were injected with 5 ml. of Strain 19 vaccine. Blood samples were taken weekly for 4 weeks, every other week for 24 weeks and monthly through 1 year. Each serum sample was tested by the standard tube, the complement fixation, mercaptoethanol and rivanol plate tests, and the 65 C heat inactivation test. Quarter milk samples from each lactating animal were tested by serial dilution milk ring tests. Results evaluated to date showed that early antibody response in animals not previously vaccinated was detectable by CF but was generally heat-, rivanol- and mercaptoethanol-

sensitive. The later antibody in these animals and in the previously stimulated ones was reactive on all tests. On the descending titer curve the CF test receded below diagnostically significant levels earlier than any other test. Evaluation of this study is continuing.

Taxonomic investigations of the cultures from 34 infected herds yielded the following distribution of types: Type I, 19; Type II, 4; Type IV, 1; Type I not requiring CO_2, 10. The value of establishing the biotype is shown in the finding that 3 of the 4 herds infected with type II were infected from a single source.

(Madison, Wisconsin) (ADP a1-32)

Under a PL 480 Grant, investigations on "The Immunizing Effect of Brucella Cell Wall" continued at the Hebrew University, Hadassah Medical School, Jerusalem, Israel.

It was previously reported that vaccines prepared from cell walls were more effective in mice than intact cells. During the past year Brucella abortus and Br. suis cell walls were fractionated by extraction with phenol into 7 fractions. The fractions obtained from Br. abortus were examined for toxicity, agglutinin binding capacity, induction of agglutinins in mice and skin reactivity in hypersensitive guinea pigs. All of the fractions were capable of binding agglutinins and of producing delayed reactions in guinea pigs, although not in the same degree. Fraction PhR_2 immunized mice very effectively, had low toxicity, induced only a mild reaction in allergic guinea pigs, and was obtained in good yield. These properties are very important for a material intended for vaccination and, therefore, fraction PhR_2 will be further examined.

(Jerusalem, Israel) (A10-ADP-6)

M. Paratuberculosis of Cattle (Johne's Disease).

The National Animal Disease Laboratory, Ames, Iowa, reported as follows:

The only laboratory technique available for identification of M. paratuberculosis was based on its requirement for the growth factor, mycobactin, which required up to 3 months to determine. Therefore, the applicability of the simpler and more rapid niacin, neutral red, and catalase tests was determined. The neutral red test was positive in those strains of M. paratuberculosis known to be virulent; it was negative in the avirulent strains. The niacin test was negative for recently isolated strains of M. paratuberculosis and for the organisms harvested directly from infected mucosa and 4 of 7 laboratory stock strains. In 3 strains, however, it was positive. In the catalase test, M. paratuberculosis reacted differently than did other species of mycobacteria. Incubation at 68 C at

either pH 7 or pH 5 generally reduced the activity but did not eliminate it. We concluded that this series of tests could be used as presumptive evidence for the identification of M. paratuberculosis. For positive identification, however, the mycobactin requirement still must be determined.

Since tuberculosis eradication programs in the United States have been based on intradermal testing, a vaccine to protect against Johne's disease should not cause skin sensitivity to tuberculin. To determine whether circulating antibodies could be produced without causing skin sensitivity at the same time, lambs were inoculated with whole cells, cell walls, and protoplasm of M. paratuberculosis. Whole cells caused lambs to have greater skin sensitivity than did cell walls. Neither skin sensitivity nor complement-fixing antibodies were found in lambs inoculated with protoplasm, but precipitating and hemagglutinating antibodies were produced in response to all 3 inoculums.

(Ames, Iowa) (ADP al-35)

N. Physiopathologic Aspects of Lupinus sericeus and L. caudatus (crooked calf syndrome).

Researchers at the Division's Poisonous Plants Laboratory, Logan, Utah, report as follows:

Deformities in calves continue to be a serious problem in many areas of the United States. One of these important congenital malformations is the crooked calf syndrome. The mortality and morbidity from this disease continues to cause livestockmen large annual economic losses. One ranch studied this year had 21.5% of the calves malformed and die because of this deformity. Varied opinions were held relative to the cause(s) and lesions associated with the crooked calf syndrome.

Deformed calves characteristic of the natural occurring cases have been experimentally induced by feeding lupine plant to pregnant heifers during various stages of gestation. The lupine plant fed was collected from areas where this disease has been a serious problem. Changes associated with this deformity vary, depending upon the stage of gestation during which the plant is fed. Based on data accumulated to date, 10th to the 40th, 40th to the 70th, and 70th to 100th days are the gestation periods during which deformed calves were induced.

Changes associated with experimentally induced and naturally occurring cases of deformed calves are being studied and characterized. These data can be useful in controlling the disease in cattle and beneficial in correlating and studying similar deformities in man.

(Logan, Utah) (ADP al-28)

Plants of the State of Sao Paulo Poisonous to Domestic Animals

Under a PL 480 Grant, the Instituto Biologico, Sao Paulo, Brazil, has developed information on a variety of poisonous plants indigenous to Brazil. Scientists there have found one species of plant, toxic for animals, that they believe had not previously been so identified.

(Sao Paulo, Brazil) (PL 480 - S3-ADP-3)

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AREA NO. 2 - INFECTIOUS AND NONINFECTIOUS DISEASES OF SWINE

Problem. Profitable swine production depends largely on the ability to control diseases. Swine diseases cause losses estimated at more than $200 million annually. In order to control and eventually eradicate these diseases, a thorough knowledge of causes, diagnostic procedures, preventative procedures, and treatments is required. Although a great deal of excellent research has been and is being accomplished, a vast amount of research is still required to obtain this knowledge. At present, the causes of several important swine diseases are unknown or incompletely understood. Extensive fundamental research on swine diseases is essential to the welfare of the swine industry.

USDA AND COOPERATIVE PROGRAM

The Department has a long history of swine disease research. For example, research on hog cholera was initiated in 1884. Research on this and other important swine diseases is a continuing long-term program. Modern research techniques in the areas of biochemistry, biophysics, pathology, microbiology, pharmacology, physiology, and immunology, are being applied to swine disease problems. Research is being conducted on the following diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 12.7 scientific man-years. This effort is divided among subheadings as follows:

Hog Cholera 5.4 at the National Animal Disease Laboratory, Ames, Iowa, and under a contract with the University of Nebraska, Lincoln.

Erysipelas 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Brucellosis 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Abscesses 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Atrophic Rhinitis 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Transmissible Gastroenteritis 2.3 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with Purdue University, Lafayette, Indiana, and the University of California, Davis.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 31.2 scientific man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Hog Cholera.

Pilot field studies to evaluate diagnostic tests, biologic products, and quarantine measure for a hog cholera eradication program. Preliminary exploratory studies were conducted on reactions of pigs to sublethal doses of virulent hog cholera virus. The objectives of these studies were (1) to establish a foundation of experimental evidence for further research on the transmission and immunizing effect of repeated, increasingly larger, but sublethal doses of virulent hog cholera virus on swine, (2) to demonstrate the variations in reactions in different swine associated with exposure or challenge exposure with minimal infecting doses of virus, and (3) to present other uses of minimal infecting doses of live virus in swine, such as for virus characterization.

Sixty-two pigs and 2 strains of virus were used. Thirty-one pigs died with signs and lesions at necropsy, usually associated with hog cholera following the first exposure to hog cholera virus. These pigs were considered to have been nonimmune (susceptible). The other 31 pigs survived the initial exposure to hog cholera virus, and were given increasingly larger, but sublethal doses of virus. Twenty-one of these also survived one or more subsequent exposures to hog cholera virus, but did not develop complete immunity. Upon exposure to greater doses of virus, they developed hog cholera and died. These pigs were considered to have been partially immune. Five of these 21 pigs, all of which had been treated with the same strain of virus, had hog cholera reactions following doses of diluted virus and recovered. Upon challenge inoculation with larger doses of virus, however, they were found not to have developed complete immunity. In other words, the hog cholera reaction mentioned in these 5 pigs did not indicate an immune response, and may have indicated a characteristic of this particular strain of virus.

Ten pigs survived the initial exposure and subsequent exposures to diluted but increasingly larger doses of virus, and developed complete immunity. This was demonstrated by their survival following subsequent challenge exposure to 1.0 ml. of undiluted, virulent hog cholera virus.

(Ames, Iowa) (ADP a2-13(Rev.))

Protective Properties of Anti-Hog Cholera Serums. Anti-hog cholera serums from 11 commercial producers, tested in graded doses against regular and variant hog cholera viruses on cholera-susceptible pigs, showed that there was a marked variation in the protective properties of these serums. The percentage of protection against regular virus varied from 47 to 100 and against variant virus from 17 to 50. The serums that gave the best protection against regular virus usually gave the poorest protection against variant virus. The smallest amount of serum per pound body weight required to protect a pig without signs of a reaction varied from 0.02 to 0.22 ml. with regular virus, and from 0.17 to 0.50+ ml. with variant virus.

(Ames, Iowa) (ADP a2-17)

Effect of TGE on CVG Immunization. Three lots of pigs on one farm were infected with transmissible gastroenteritis (TGE) at different ages, and later vaccinated with crystal violet glycerol (CVG) vaccine. One month later, a second dose of vaccine was given to some of the pigs in each lot. Five months after the first injection, pigs from each lot were given virulent hog cholera virus.

The youngest age lot protected from TGE by the colostrum, and the older age lot protected by having had TGE, were better protected by a single dose of CVG vaccine than the middle age lot that was actively infected with TGE at time of vaccination; however, none of these was adequately protected. The percentage of protection for the pigs given 1 dose of CVG vaccine at 2, 6, and 10 weeks of age was 43.0, 14.8, and 58.8, respectively. The percentage of protection for these same lots of pigs given 2 doses of vaccine was 70.4, 74.6, and 79.8, respectively. The first dose of vaccine made the pigs more receptive so that a second dose produced adequate protection.

(Ames, Iowa) (ADP a2-17)

Pathogenicity of Field Viruses. Specimens from 339 field cases of suspected hog cholera were collected in 23 states. Of this number, 332 were tested in cholera-susceptible pigs to determine the nature of the viruses involved. Viruses sufficiently pathogenic to kill the pigs were demonstrated at necropsy in 190 cases. In 53 cases, the virus was sufficiently pathogenic to make the pigs sick, but they recovered. Of the 53 cases, 33 immunized the pigs, and in 20 cases, the pigs died after challenge exposure. In 89 cases, pigs given the specimens did not get sick. In 76 of these cases, the pigs died after challenge exposure with virulent hog cholera virus, and in 13 cases, the pigs were immune.

(Ames, Iowa) (ADP a2-17)

Inability to Distinguish the Sex of Swine by Studying Kidney Cells Grown in Tissue Culture. In many animal species, the female possesses certain characteristic objects in the nuclei of her cells, whereas the male counterpart does not. This characteristic is apparently not true for swine. Therefore, cells from both male and female swine were grown in tissue culture, and microscopic examinations were made on the cultures to identify the "sex chromatin" in the nuclei. It was determined that the incidence of sex chromatin was approximately the same in both male and female swine cells, thus not allowing for determination of sex by this method.

(Ames, Iowa) (ADP a2-17)

Direct Fluorescent Antibody Technique for Diagnosis of Hog Cholera. The direct fluorescent antibody test for the diagnosis of hog cholera was developed under contract with the University of Nebraska. It has proved to be rapid, accurate, specific, and sensitive. It is a technique that can be readily adapted in any laboratory equipped to conduct fluorescent antibody procedures, and is proving of value as an important tool in the eradication of hog cholera in the United States. A brochure is in preparation outlining the procedure for performing this test. The contract was completed during the year. A new contract was awarded the University to study the use of the direct fluorescent antibody test to diagnose hog cholera when the disease results from viruses of low virulence. The research is designed to find out if the test can locate hog cholera in disease outbreaks where no one, including veterinarians, suspects that hog cholera is the problem.

(Lincoln, Nebraska) (ADP a2-17)(C))

B. Swine Erysipelas.

To improve control of swine erysipelas, detailed knowledge is needed on how the disease is spread. An efficient and simple method for cultural detection of the causative organism from heavily contaminated materials, such as feces and urine, was developed, tested, and found satisfactory. The medium, containing high concentrations of the antibiotics kanamycin, neomycin, and vancomycin, permitted consistent detection of the erysipelas organism in pig feces having 6 to 11 live cells per gram. This number was about 100th that which was consistently detectable without the antibiotics.

In previous studies, significant changes in blood glucose levels and serum glutamic oxalacetic transaminase (SGO-T) activity occurred in pigs affected with acute erysipelas. However, it is known that starvation also causes changes is blood glucose levels and SGO-T activity. Thus, the reduced intake of feed from inappetence of the pigs when acutely ill may have been a factor in the changes reported in swine erysipelas. Experiments were conducted in which blood glucose levels and SGO-T activity in pigs affected with acute erysipelas were compared to the same parameters in fasted pigs.

Blood glucose decreased to minimums of 40.6% of normal in pigs that died of acute erysipelas and 60.8% of normal in those that became ill but recovered from the disease. In fasted pigs (not infected), blood glucose decreased to 85.8% of normal. SGO-T activity increased to 700% of normal in pigs that died of erysipelas and 267% of normal in those that recovered. In fasted pigs (not infected), SGO-T activity decreased to 82.8% of normal.

It was concluded that the decrease in blood glucose levels during acute swine erysipelas was caused by: (1) undetermined physiologic factors associated with the disease, and (2) to a lesser extent by reduced feed consumption. The levels of SGO-T activity increased significantly in the exposed pigs. However, the highest level reached was much lower than that usually associated with extensive tissue damage.

(Ames, Iowa) (ADP a2-15 and ADP a2-21)

C. Swine Brucellosis.

Studies have been in progress to determine the location, incidence, and characteristics of lesions produced by Brucella suis in bones of 4-month-old pigs. Also, because of the recognized need of regulatory officials for more definitive tests for the diagnosis of swine brucellosis, various standard and supplemental tests are being conducted on serums collected from experimental swine.

(Ames, Iowa) (ADP a2-16)

D. Swine Abscesses.

Each of 3 strains of Lancefield's group E streptococci were fed to a group of specific-pathogen-free pigs. One of the strains induced the formation of abscesses in 5 of 5 pigs; no abscesses were found in the other groups of pigs attributable to group E streptococci. Four of the 5 abscessed pigs had cutaneous reactions after the intradermal injection of 0.1 ml. of cell-free culture filtrate of 5 strains of group E streptococci. Except for 1 pig, no reactions to these filtrates were observed in the nonabscessed pigs. In the precipitin test, the serums of 4 of the abscessed pigs reacted with the homologous filtrate antigen, and 2 of the 4 pigs reacted with the filtrate antigen of another group E streptococcus. No serums of pigs with abscesses reacted with extracts prepared from the bacterial cells of 5 strains of group E streptococci. No significant rise in the serum agglutination titer of pigs with abscesses were observed. The results of the intradermal and precipitin tests furnish a lead to the possible development of a diagnostic test.

(Ames, Iowa) (ADP a2-19)

E. Atrophic Rhinitis.

Research on atrophic rhinitis (AR) of swine was reinitiated last year. The research is directed to solving basic problems of raising specific-pathogen-free, hysterectomy-derived pigs, developing tissue culture cell lines, and developing isolation and identification procedures for bacteria. These various techniques and methods will be used as tools to identify the causes of AR.

(Ames, Iowa) (ADP a2-20)

F. Transmissible Gastroenteritis.

The effect of antiviral drugs on viruses associated with transmissible gastroenteritis (TGE). The effect on the plaque production of a cytopathogenic virus, a myxovirus, from transmissible gastroenteritis of swine, by 5-bromo-2'-deoxyuridine (BUDR), 5 iodo-2'-deoxyuridine (IUDR), actinomycin-D, puromycin, and amantadine-HCl (Symmetral) has been studied.

Symmetral reduced the plaque-forming units of virus per ml. by approximately 98%. Puromycin prevented almost all virus reproduction while actinomycin-D caused approximately a 30% reduction. Both IUDR and BUDR produced a 20% increase in plaque-forming units of virus per ml.

Swine testis cells stained with acridine orange early in the course of infection contained brick-red particles in the cytoplasm, indicative of an RNA virus.

(Ames, Iowa) (ADP a2-22)

Characterization of Viruses Associated with Transmissible Gastroenteritis. Research efforts are being directed to purifying and characterizing different viruses isolated from gastroenteritis-infected intestinal tissue. Attempts will be made to evaluate the role that Mycoplasma organisms play in the disease.

(Ames, Iowa) (ADP a2-23)

At the University of California, cooperative studies were conducted on two new enteroviruses isolated from swine from California herds with intestinal disorders, and a means for a rapid diagnosis of transmissible gastroenteritis (TGE), confirmed for the first time in California.

The L strain of virus was isolated as a cytopathic plaque-forming agent (CPE) in pig kidney (PK) cells inoculated with a sample of intestinal contents from a pig with TGE from the California herd. The virus was separated from TGE virus by a limiting dilution technique and by selecting and subpassaging the progeny of a single plaque in cell culture overlayered with agar containing TGE immune serum. To eliminate the possibility of contamination with TGE virus, a suspension of L virus was inoculated into specific-pathogen-free, colostrum-deprived (SPF) pigs that had no signs of disease.

(Davis, California) (ADP a2-23)

In cooperative studies at Purdue University, a tissue culture-adapted strain of transmissible gastroenteritis (TGE) virus from Japan was characterized, and should prove very useful in further studies on the nature of the virus and on the pathogenesis and immunity to TGE. Using this virus, a practical and reliable immunological test is now available that may be used for epidemiological work.

A characterization of changes in blood constituents during the course of TGE was accomplished. This characterization is primary for developing replacement therapy to combat the dehydrating effects of TGE diarrhea.

Additional progress was made in studying the mechanism of transfer of TGE immunity from sows to pigs. The concept of this mechanism, which has been termed "lactogenic immunity," is new and not completely understood. The development and evaluation of vaccines against TGE will depend upon recognition and use of the results of basic research on this mechanism.

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AREA NO. 3 - INFECTIOUS AND NONINFECTIOUS DISEASES OF SHEEP AND GOATS

Problem. Infectious diseases of sheep and goats in the United States cause an estimated annual loss of 15 million dollars. Noninfectious diseases are estimated to cause an additional 3 million dollar loss annually. The cause of some of these diseases is known; others have more than one causative agent contributing to produce the effects seen in field cases. Environmental, genetic, and unknown factors appear to play a part in some diseases. The natural reservoirs of the known infectious agents have not been fully determined. Fundamental information on methods of transmission and means of prevention are needed for many of these diseases. Vaccines and other immunizing products are available for some diseases of sheep but not for others. Some of these products might be improved. Prevention, control, or eradication of disease is necessary for economic and efficient sheep and goat raising. Because of lack of accurate, rapid diagnostic techniques, infectious diseases often get a substantial start in a band or flock before they are recognized, partly because they are easily confused with noninfectious diseases.

USDA AND COOPERATIVE PROGRAM

The Department has a continuous long-term program involving veterinarians, biochemists, microbiologists, and pathologists engaged in both basic studies and the application of known principles to the solution of infectious and noninfectious diseases of sheep and goats. Research is being conducted on the diseases at the following designated locations.

The Federal scientific effort devoted to research in this area totals 7.5 scientific man-years. This effort is applied as follows:

Bluetongue 4.0 at the Animal Disease Research Laboratory, Denver, Colorado.

Vibriosis 0.2 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the Colorado, Montana, and Utah Agricultural Experiment Stations.

Scrapie 0.2 at the Agricultural Research Council Field Station, Compton, Berkshire, England, and the Moredun Institute, Edinburgh, Scotland, through two grants of PL 480 funds.

Paratuberculosis 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Ulcerative Dermatosis 0.1 under a cooperative agreement with the Colorado Agricultural Experiment Station, Fort Collins.

Toxicological Effects of Oxalate-Containing Plants 1.0 at the Poisonous Plants Research Laboratory, Logan, Utah.

Identification of Teratogenic Agent in Veratrum californicum 0.5 at the Poisonous Plants Research Laboratory, Logan, Utah.

Chronic Toxicity of Herbicide Accumulation in Sheep Tissues 0.5 at the Poisonous Plants Research Laboratory, Logan, Utah.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 25.8 scientific man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Bluetongue .

At the Division's Animal Disease Research Laboratory at Denver, Colorado, the following work was reported:

Observations on Bluetongue Virus in the Salivary Glands of an Insect Vector, Culicoides variipennis. The salivary gland of the non-blood fed fly is apparently a syncytium, since no discrete intercellular membranes were observed, and the nuclei were scattered throughout the lighter staining cytoplasmic areas. About 80 percent of the gland was occupied by large, dark-staining masses.

Bluetongue virus reproduces (replicates) within, or in close association with, inclusion bodies found in the light-staining portion of the large dark-staining masses seen in the glands of all non-blood fed flies. The membranous elements of the gland underwent extensive vesiculation, and the viral RNA became associated with these vesicles. The virus particles do not have an envelope on the interior of the inclusion body, but have obtained the typical BTV envelope when they were observed in the lighter portions of the cytoplasm.

This evidence is an important step in establishing how insects transmit virus diseases.

Current Aspects of Bluetongue in Cattle. Bluetongue disease is caused by a filterable virus 50 mμ in diameter. Bluetongue virus was isolated from cattle on a feed lot and from a dairy herd in close proximity to a bluetongue epizootic in sheep. The sheep were shipped into the area and were observed to be infected 10-14 days later. The disease vectors were, therefore, infective and available to infect susceptible hosts. The cattle had no apparent disease; however, they had the virus in their circulating blood.

Bluetongue has been transmitted experimentally from sheep to cattle and cattle to sheep by Culicoides variipennis; however, the cattle had no outward signs of disease while the virus was readily isolated from their blood stream.

Bluetongue in cattle has been isolated and confirmed from six western states. The field veterinarians report that these animals had signs and symptoms similar to those seen in BTV-infected sheep; however, the same virus caused no signs of disease when injected into experimental cattle held under strict environmental isolation. BTV was isolated on a weekly basis up to 49 days after artificial infection. Specific BTV neutralizing antibodies were also present when the virus was isolated from the blood stream.

A diagnosis of BT disease cannot, at present, be made in the field but must be confirmed by laboratory tests of isolation of the virus.

Cytopathology and Development of Inclusion Bodies in Cultured Cells Infected with Bluetongue Virus. Bluetongue virus (BTV) causes cytopathogenic lesions in cell cultures, including the development of two different types of intracytoplasmic inclusion bodies. Type I inclusion bodies originate from the perinuclear space within the nuclear envelope. Material accumulates within this space and eventually pinches off from the outer nuclear membrane, resulting in an intracytoplasmic body bounded by a single membrane. No BTV synthesis was found associated with this type of inclusion body. Evidence was obtained to support the intranuclear origin of Type II inclusion bodies. They apparently escape the nuclear envelope through much enlarged pores. Type II inclusion is RNA-positive in the cytoplasm and DNA-positive in the nucleus. It fluoresces in response to specific BTV conjugate when it is intracytoplasmic; however, it was not observed to fluoresce specifically in the nucleus. Although no evidence of viral structure was observed in the nucleus of BTV-infected cells studied in the electron microscope, mature BTV was often observed in association with Type II intracytoplasmic inclusions.

Transmission of Bluetongue Between Sheep and Cattle by Culicoides variipennis. Fourteen positive biological transmissions of bluetongue were obtained when the biting midge, Culicoides variipennis (Coquillett), were incubated 10 to 16 days after feeding on bluetongue-infected sheep or cattle and then allowed to take a second blood meal on a susceptible animal. Five of the transmissions occurred from cattle to sheep, 5 from sheep to sheep, 3 from sheep to cattle, and 1 from cattle to cattle. Cattle developed a minimal clinical response to bluetongue infection but were proved infected by virus isolation. In addition, they developed a level of serum neutralizing antibodies of the same magnitude as in sheep, and they were resistant to a challenge inoculation of their immunity with the homologous virus.

The Transmission of Bluetongue Virus to Embryonating Chicken Eggs by Culicoides variipennis (Diptera: Ceratopogonidae) Infected by Intrathoracic Inoculation. Experiments with the biological transmission of bluetongue disease of sheep were conducted with the biting midge, Culicoides variipennis (Coquillett). All flies were infected artificially by intrathoracic inoculation of egg-adapted bluetongue virus. Embryonating chicken eggs were used, both as the recipient host in transmission experiments and as the assay system for the detection of virus.

The infection rate was 100 percent. The transmission rate was zero percent for 1-2 days of incubation of the fly after infection. It became 100 percent at 6 days of incubation, and with the exception of two negative transmission experiments at 21 days, continued at 100 percent throughout the remainder of the 28 days of the test. In a paired experiment for 1-7 days of incubation, wild and colony flies had equivalent transmission rates. In the course of the experiments it was found that the probe of a fly, with no detectable meal, was also sufficient for biological transmission.

(Denver, Colorado) (ADP a3-5)

B. Vibriosis in Sheep .

Research under a cooperative agreement with the Colorado Agricultural Experiment Station was continued. Investigations were carried out to determine the duration of immunity in ewes vaccinated as yearlings in 1963 with V. fetus serotypes I and V oil adjuvant bivalent bacterin. Challenge exposure was made with the combined V. fetus serotypes I and V organisms at 4 years of age during advanced gestation of their third pregnancy.

A group of 69, four-year-old pregnant ewes, arranged in lots 7, 8, and 9 gave the following results when their immunity was challenged: Lot 7 -- 24 ewes, nonvaccinated, challenged -- 14 vibrionic abortions; Lot 8 -- 23 ewes, vaccinated, challenged -- 1 vibrionic abortion; Lot 9 -- 22 ewes, nonvaccinated, nonchallenged controls -- 0 abortions.

From data obtained in the 1966 duration of immunity studies, 4-year-old ewes vaccinated as yearlings had strong immunity against challenge exposure with the combined V. fetus serotypes I and V given during advanced gestation of their third pregnancy.

(Fort Collins, Colorado) (ADP a3-11)

In cooperation with the Montana Agricultural Experiment Station, work has continued on ovine vibriosis. A selective medium for the isolation of V. fetus from contaminated materials, which is completely satisfactory, has not yet been found. At the present time the following antibiotics are being used in agar containing 10 percent of bovine blood: bacitracin 10 units/ ml. of agar, polymixin-B 30 units/ ml. of agar, and actidione 100 mg./ml. of agar.

In spite of these rather high antibiotic levels, there is still considerable contamination, particularly when semen is cultured. Brilliant green at a concentration of 1:40,000 seems to have some value in retarding motility of some of the "spreaders," but there is danger that it may inhibit to a slight extent the growth of V. fetus. It is important to prepare the plates the day before use to permit adequate drying.

A bacterin for immunization against bovine vibriosis was first tested in 1963 and extensively field tested in 1964. Vaccinated cows had a conception rate of 91.5% as compared to 45.5% in the controls.

Recent work in this laboratory seems to be confirming the findings that there are at least three distinct whole cell antigens in addition to Montana serotype I.

(Bozeman, Montana) (ADP a3-11)

In cooperation with the Utah Agricultural Experiment Station, studies were made on the effect of vaccination of yearling replacement ewes with commercial Vibrio fetus vaccine containing serotypes I and V organisms to prevent vibriosis. These studies were carried out for the sixth consecutive year in 2 herds with approximately 2000 ewes each. In herd A, no V. fetus organisms were isolated from 54 aborted or stillborn lambs or from 95 placentas taken from ewes having apparently normal parturitions. In herd B, V. fetus was isolated from 1 aborted lamb out of 52 and from 1 placenta out of 100.

All the ewes in herd A were also vaccinated for enzootic abortion of ewes but those in herd B were not. Yet the amount of psittacosis-lymphogranuloma virus (PLV) in aborted or stillborn lambs was nearly the same in both herds; 20% in herd A and 29% in herd B; also, the amount of PLV-infected placentas was 24% and 25% respectively.

The clearance rate of V. fetus from the blood was studied using V. fetus serotype I organisms with coccoid and comma shape morphology and serotype V organisms with comma shape. Half the experimental ewes were immune to serotype I organisms. The enhancement of the clearance rate of V. fetus from the blood is a function of the immune state of the ewe and not of the cell morphology of the organism.

(Logan, Utah) (ADP a3-1 (Rev.))

C. Scrapie.

Scrapie was first diagnosed in the United States several years ago. It is, however, not considered to be firmly established and efforts are continuing to eradicate it. Research has been conducted on this disease in Scotland and Great Britain since 1961. The USDA is supporting this research through PL 480 grants. New grants were awarded during this year. In recent years, it has been determined that the disease is probably caused by a transmissible agent. The agent has, however, not been isolated nor characterized in detail. There is also increasing evidence that a certain genetic constitution is existent which determines susceptibility.

(Scotland E29-ADP-4) (England E29-ADP-5)

In one set of studies serums are being examined from sheep, goats, and mice which have been inoculated with the scrapie agent. The serums have been obtained from these animals at varying periods after inoculation with the scrapie agent and are being assayed for antibody by complement fixation. The experiments with sheep and goats are still in progress. Blood samples are taken monthly from the inoculated animals.

(Greenport, New York) (ADP a3-12)

D. Paratuberculosis of Sheep.

A study on the pathogenesis and cytochemical alterations occurring in sheep infected with Mycobacterium paratuberculosis is in the process of being completed.

(Ames, Iowa) (ADP a3-6)

E. Ulcerative Dermatosis of Sheep.

In cooperative research with the Colorado Agricultural Experiment Station clinically ulcerative dermatosis of sheep has been recognized, but the causative agent(s) and pathogenesis have not been clarified. The objective is to reproduce the disease in sheep and to identify the causative agent(s).

For reproducing the disease, infectious materials were obtained from 3 field outbreaks which involved hundreds of sheep in 3 different areas in Colorado. Seven normal lambs raised on the university research farm were used for reproducing the disease with the infectious materials collected from the field outbreaks. Pustules were reproduced, but no deep ulcerative lesions as seen in the field. Succeeding passages are underway.

For identification, causative agent(s)--both bacterial and viral nature--were studied. Staphylococcus, Streptococcus, Pseudomonas, and Corynebacterium were isolated and also used in combinations of inoculations on sheep. Tissue cultures, both of lamb and calf origins, and chicken embryos were used for isolation of viral agent(s). No agent has been found, but investigation is still being continued.

(Fort Collins, Colorado) (ADP a3-4 Rev.)

F. Toxicological Effects of Oxalate-Containing Plants.

Sublethal amounts of oxalate were fed to lambs over an extended period of time. The lambs receiving the oxalate drank much more water. This was considered to be due to the presence of an osmotic diuretic. This suggests that the consumption of oxalate may have a marked effect on feed consumption under certain conditions. This may be associated with acute halogeton poisoning. The lambs receiving the oxalate had a more positive calcium and potassium balance, contrary to expectations. However, these lambs had a lower plasma calcium. Oxalate crystals were found in the kidneys of the lambs fed oxalate, which indicated that some oxalate was

absorbed. This condition could be a problem to sheep being fed oxalate over more extended periods.

The consumption of oxalate resulted in heavier liver weight, and a trend toward heavier weight in heart, spleen, and kidneys.

There was no difference in carcass composition, but there was a trend toward higher bone potassium in the lambs receiving oxalate.

There was no difference in carcass weight.

(Logan, Utah) (ADP a3-7)

G. Identification of Teratogenic Agent in Veratrum californicum .

Veratrum californicum produces a congenital cyclopian-type malformation when fed to pregnant ruminant animals such as sheep, cows, and goats. The feeding of this plant to pregnant nonruminant animals such as rabbits and swine has failed to produce any such congenital malformation. Possibly the compound in the plant, associated with the malformation, is altered by the rumen flora and made teratogenic.

Ewes fed Veratrum californicum from the 30th through the 32nd day of gestation gave birth to lambs with a marked shortening of the metacarpal and metatarsal bones.

The time of insult in the congenital cyclopian-type malformation caused by V. californicum was shown to occur primarily between 14 days and 14 days 9 hours after breeding. This period indicates that the teratogenic compound is highly specific in its action and also that the affected tissue is susceptible for only a very short period of time.

Astragalus miser (timber milk vetch) is poisonous to both cattle and sheep. It is about 2 1/2 times more toxic to cattle than sheep and also more toxic to cattle than Delphinium barbeyi (tall larkspur).

In cattle, the toxic signs of A. miser and D. barbeyi poisoning are very similar and both are very toxic. Therefore, care must be taken in diagnosing plant poisoning in livestock in areas where these two plants grow.

(Logan, Utah) (ADP a3-8)

H. Chronic Toxicity of Herbicide Accumulation in Sheep Tissues .

The herbicides Atrazine [2 chloro-4, ethylamino-6, isopropyl-amino-S-triazine] and Monuron [3-(4-chlorophenyl)-1, 1-dimethyl urea] were each fed to pregnant ewes throughout the gestation period. Chemical analysis of the tissues of the ewes and lambs for the herbicide-fed showed that there was no significant accumulation of either herbicide.

(Logan, Utah) (ADP a3-9)

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AREA NO. 4 - DISEASES AND PARASITES OF HORSES

Problem. Currently there are about 3,250,000 horses in the United States, valued at approximately $860 million. About one million of these are draft animals. Considerable numbers of horses and mules are still required for work on cattle ranches and as pack animals. The annual overall value of the horse industry has been estimated at about $1.5 billion. The horse may be an important link in epizootiology of animal diseases in general. Equine piroplasmosis is an acute, subacute, or chronic tick-borne disease of horses caused by protozoan parasites that was first recognized in this country in Florida in 1961. It is characterized by high fever, progressive anemia, jaundice, edema, extreme weakness, and depression. Fatalities range from 5 to 50 percent of infected animals. This disease, now apparently well established in Florida, has extended into Georgia and poses a serious threat to the entire equine population in the southern United States. The disease is clinically indistinguishable from equine infectious anemia. Horses that have clinically recovered from piroplasmosis usually remain carriers of the disease and are a potential source of infection. African horsesickness, a highly fatal disease of equine animals, that was confined to Africa has caused serious losses in the Middle East and parts of Asia in recent years.

USDA AND COOPERATIVE PROGRAM

The Department has a program involving biochemists, pathologists, protozoologists, and veterinarians working on equine piroplasmosis. In order to be prepared in the event of introduction of African horsesickness into the United States, the Plum Island Animal Disease Laboratory has obtained African horsesickness viruses and antiserums from South Africa. These materials are thus directly available for diagnostic and vaccine studies should the need arise.

The Federal scientific effort devoted to research in this area is 2.0 scientific man-years. This effort is divided among sub-headings as follows:

Serological Diagnosis, Transmission, and Control of Equine Piroplasmosis 2.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland (in cooperation with the Entomology Research Division), and under contracts with the University of Florida, Gainesville, and the University of Kentucky, Lexington.

PL 480 funds have been made available in Turkey for research on Gastrophilus pseudo-hemorrhoidalis (equine parasite) in Turkey, and for the study of the horsesickness virus.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 11.5 scientific man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Equine Piroplasmosis.

Evaluations of serologic diagnostic tests, principally, the complement-fixation, the fluorescent-antibody, and the agar-gel double diffusion precipitin techniques are being continued. The relationships of the results of these tests to the "carrier state" are being determined. Studies of fixed stained blood films and observation of living parasites in freshly drawn blood are being continued to ascertain more fully the developmental cycles in the horse. These studies are being made with both Babesia caballi and Babesia equi. Larvae of ticks (Dermacentor nitens), obtained from the enzootic area of Florida, transmitted B. caballi to horses on 5 separate trials.

(Beltsville, Maryland) (ADP b6-13)

Research studies under a contract with the University of Florida were completed during the reporting year. Also during the year, a new contract was awarded the University for studies aimed at identifying drugs useful in preventing, treating, and eradicating piroplasmosis in horses. Last year, piroplasmosis that was caused by Babesia equi was discovered in Florida. Because the same drugs and dosages of drugs found valuable in treating horses infected with Babesia caballi are not effective against B. equi, further research is needed. Three drugs, Phenamidine, Berenil, and Diampron, were found beneficial in the treatment of B. caballi infections. Phenamidine was used in treating 38 experimental cases of piroplasmosis. Berenil was used for 29 experimentally infected horses and Diampron for 41 experimentally infected horses.

(Gainesville, Florida) (ADP b6-13(C))

During the past 6 months, 35 laboratory animals were given B. caballi-infected pony red blood cells in an attempt to establish infection in these animals. Within the past 10 months, 186 laboratory animals have been inoculated with infected pony cells. The species of laboratory animals included: mice, hamsters, rabbits, guinea pigs, cats, dogs, and sheep. No evidence of B. caballi being established in any of these animals has been found, based on direct blood smears and microhematocrit determinations.

One pony developed a 640 complement-fixation titer after having received rabbit blood from rabbits that had been given infected pony blood. This pony was not immune to piroplasmosis and, based on subinoculation of his blood into a splenectomized pony, was not a carrier. This titer is as yet unexplained.

The blood has been collected from two terminal piroplasmosis cases for antigen production. Capability to run the complement-fixation test for piroplasmosis is being developed.

(Lexington, Kentucky) (ADP b6-13(C))

B. Gastrophilus pseudo-hemorrhoidalis (horse bot fly).

In Turkey under a PL 480 grant, a total of 662 equine animals (475 horses, 173 donkeys, and 14 mules) was checked for Gastrophilus pseudo-hemorrhoidalis larvae through rectal examination. A total of 1017 larvae of different Gastrophilus species was collected. Of these, 539 were G. intestinalis, 295 G. hemorrhoidalis, and 183 G. nasalis. None represented G. pseudo-hemorrhoidalis. The larvae of G. pseudo-hemorrhoidalis supposedly differ from the other species of the genus in that they spend many months attached to the wall of the rectum. The purpose of this study is to establish the salient facts concerning its life cycle.

(Ankara, Turkey) (A22-ADP-4)

C. African Horsesickness Virus .

Under a PL 480 grant to the Veterinary Faculty, University of Ankara, Ankara, Turkey, studies of horsesickness virus in tissue cultures, its serological and immunological characteristics, and its vectors, have continued. Seven vaccine strains were propagated in mouse cells and carried for a number of passages. Field virus was propagated in baby hamster and embryonic horse kidney epithelial cells but could not be propagated in rabbit and mouse kidney cells. No cytopathogenic effect was produced in mouse cells at pH 7.2 and the virus titer did not exceed 10^{-3}. At pH 7.4, however, cytopathogenic effects began 2 hours after inoculation and the cells detached from the flask at 72 hours. Virus titers of 10^{-5} were attained, which indicated that the pH range for virus propagation is from 7.2 to 7.5.

(Ankara, Turkey) (A22-ADP-7)

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None

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AREA NO. 5 - INFECTIOUS AND NONINFECTIOUS DISEASES OF POULTRY

Problem. Annual losses from infectious and noninfectious diseases of poultry, exclusive of parasitisms, are estimated to be at least $200 million. Continued and expanded basic and applied research are essential to aid in reducing these losses, which inevitably affect cost to the consumer. Added to the initial losses from mortality, reduced weight gains, poor feed utilization, decreased egg production, and lowered quality, are the final losses occasioned by condemnations at dressing plants. United States turkey growers in particular, are faced with a new problem in that a newly discovered infection with a different strain of Mycoplasma is widespread in flocks throughout the country. Resulting condemnation losses at slaughter are often great. The problem is to keep abreast of changing conditions in the field, which present increasingly complex problems requiring basic information.

USDA AND COOPERATIVE PROGRAM

The Department has a long-term program involving biochemists, microbiologists, pathologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of infectious and noninfectious diseases of poultry. Research is being conducted on the diseases at the following locations.

The Federal scientific effort devoted to research in this area totals 16.5 scientific man-years. This effort is applied as follows:

Ornithosis 2.7 at the National Animal Disease Laboratory, Ames, Iowa.

Salmonellosis 1.0 at the Southeast Poultry Research Laboratory, Athens, Georgia.

Pasteurellosis 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Chronic Respiratory Disease Complex 5.3 at the National Animal Disease Laboratory, Ames, Iowa, the Southeast Poultry Research Laboratory, Athens, Georgia, and under cooperative agreements with the Agricultural Experiment Stations of Georgia, North Carolina, and Wisconsin, and with the University of Minnesota.

Newcastle Disease 3.2 at the National Animal Disease Laboratory, Ames, Iowa, the Southeast Poultry Research Laboratory, Athens, Georgia, and under cooperative agreements with the University of Maine and the Wisconsin Agricultural Experiment Station, and under a PL 480 Grant to the Institute for Veterinary Research, Pulawy, Poland.

Leukosis 0.3 under cooperative agreement with the Regional Poultry Research Laboratory, USDA, East Lansing, Michigan.

Infectious Bronchitis 2.0 at the National Animal Disease Laboratory, Ames, Iowa, and the Southeast Poultry Research Laboratory, Athens, Georgia.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 85.3 scientific man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chronic Respiratory Disease Complex.

At the Southeast Poultry Research Laboratory, Athens, Georgia, the effect of various temperatures of pre-incubation storage or treatments of hatching eggs was studied to determine the possible use of such procedures to reduce or eliminate egg transmission of Mycoplasma gallisepticum (PPLO). The storage of eggs at lower than a normal holding temperature for 1 week reduced, but did not eliminate, egg transmission. Preliminary studies indicate that elevated pre-incubation temperature for a short period of time may be more effective than prolonged cooling.

Various combinations of ingredients were studied to devise more adequate mediums for the isolation and cultivation of most serotypes of avian Mycoplasma including M. synoviae. Tryptose phosphate broth was adequate when supplemented with swine serum and possibly yeast autolysate. Selected serotypes are being adapted to medium containing rabbit serum so that antigen preparations can be obtained to inoculate rabbits for producing high quality antiserums for serotyping studies.

Attempts to reproduce a mild respiratory infection with only M. gallisepticum inoculum in young chickens in isolation units indicates that unfavorable environmental conditions are needed to enhance the severity of the infection after either intratracheal or aerosol exposure.
(Athens, Georgia) (ADP a5-29)

At the South Central Poultry Research Laboratory, State College, Mississippi, investigations were instigated to study the effects of insulation versus noninsulation, density, temperature fluctuation, and nursery versus no nursery on condemnations. This study was made from October 16, 1965, to December 15, 1965. In each pen 1% of the birds were intranasally infected with M. gallisepticum at 1 day of age. Statistical analysis did not show any significant condemnation differences on the nursery versus no nursery. Pens that had 0.7 square foot space per bird had a significantly higher condemnation rate from airsacculitis and from total farm causes than birds

that had 1.0 square foot/bird. There were no significant differences in the percentage of condemnations when comparing the 2 temperatures (70° F after the third week versus fluctuating temperatures after the third week).

In another trial, December 29, 1965, to February 24, 1966, 3% of the birds in each pen were intranasally infected with M. gallisepticum and comparisons were made on nursery versus no nursery, insulation versus no insulation with sexes equally mixed and insulation versus no insulation with sexes separated.

The percentage points difference from condemnations from (1) airsacculitis, and (2) total farm causes gave a highly significant difference (P.01) among the following groups:

1. Insulation* versus no insulation (all birds)

2. Insulated nursery versus insulated no nursery

3. Insulated nursery versus noninsulated nursery

4. Males (2 pens of insulated and 2 pens noninsulated) versus females (2 pens insulated and 2 pens noninsulated)

5. Insulated males versus noninsulated males

6. Insulated females versus noninsulated females

The following were significant at P.05 level:

1. Noninsulated no nursery versus noninsulated nursery

2. Insulated males versus insulated females

3. Noninsulated males versus noninsulated females

* The group underlined had the highest condemnation rate.

The total condemnations from farm causes were highly significant in the nursery over the no nursery, and condemnation from air sac was significant.

In 2 trials to determine the spread of M. gallisepticum from 1 infected pen to other pens in the same house, 1% of the birds in 1 of 4 pens in a square (pen 2) were intranasally infected with M. gallisepticum at 1 day of age. Blood secured at the processing plant and M. gallisepticum serum plate tested was 100.0% positive in pen 2, the infected pen; 17.78% and

12.23% positive in the 2 adjoining pens (1 and 4, respectively); and 3.79% positive in pen 3. The total farm condemnation rate at the processing plant was 2.97% in pen 2, the infected pen; the two adjoining pens--1 and 4--were 1.55 and 0.854%, respectively, and pen 3 was 0.902%.

(State College, Mississippi)(ADP-a5-29)

In cooperative research at the Raleigh, North Carolina Agricultural Experiment Station, a standardized procedure has been developed for the production of a uniform, high titer live culture inoculum of Mycoplasma gallisepticum for use in "experimental planned infection" of broiler breeders for the control of "air sac disease" in broiler progeny.

A comparison of breeder hen and broiler performance strongly suggests eradication of Mycoplasma gallisepticum as the route-of-choice for the broiler industry, with "experimental planned infection" and natural exposure being inferior methods for reducing air sac condemnation losses in broiler production. Extensive natural exposure, occurring early in the life of the breeder parent, appears to have a greater influence in reducing air sac condemnations in broilers than planned infection. However, early natural infection resulted in higher mortality in the growing breeders and lower egg production than did planned infection.

(Raleigh, North Carolina) (ADP a5-29)

In cooperative work at the University of Minnesota, field investigations were conducted on all known and suspected outbreaks of infectious sinusitis during the past year. The primary cause of this disease in Minnesota turkeys last year was contaminated vaccines. An estimated 25,000 breeder hens and 400,000 grower birds were infected from this source.

Some 3,625 serum samples from 97 flocks were checked for the presence of antibodies to the ornithosis agent. Seven flocks (7.2%) gave positive reactions; 11 flocks (11.3%) were suspects. The remainder were serologically negative. Two human cases of ornithosis were reported in poultry processing plant employees.

Three experiments were conducted in an effort to raise "Mycoplasma-free" fryer-roaster turkey flocks. Use of the first eggs laid in a flock, along with an egg dipping and water medication program resulted in the production of 2000 "Mycoplasma-free" Broad white turkeys. Air samples taken throughout these experiments continued to indicate a high microbial count after the first 4 weeks. This high count has not had a known adverse effect on an intact respiratory system.

The voluntary control program for Mycoplasma gallisepticum continues to be effective in minimizing this infection in potential breeder turkeys in Minnesota. Only 134 birds of 764,311 birds tested were submitted to the

university for further laboratory analysis. Of these, only 1 bird was found to be infected.

The survey to determine the incidence of Mycoplasma meleagridis in Minnesota potential breeder turkeys was continued. Some 56% of all samples tested (14,075) reacted to the serum plate test antigen developed at the University of Minnesota.

Egg dipping and water medication with tylosin were used to produce a second Mycoplasma gallisepticum-free flock of chickens. This flock was 100% tested at the end of the laying season and was negative.

Two small flocks of "Mycoplasma-free" turkeys are being maintained at the Rosemount Experiment Station. Progeny of one of these flocks is in its fourth season and the other in its third. Day-old poults from these flocks are free of airsacculitis observed in Mycoplasma meleagridis-infected flocks.

The effect of a double egg dipping procedure for the control of avian Mycoplasma was investigated. The double dipping procedure was found to significantly enhance the amount of tylosin absorbed into the egg without adversely affecting the hatchability of the egg.

A salvage program for two valuable turkey breeding flocks infected with Mycoplasma gallisepticum was tried. The results of this program were variable. In hatchery A, 20 hatches involving approximately 700,000 eggs remained free. However, later in the season, poults from approximately 550,000 eggs showed clinical and serological evidence of infectious sinusitis. The reason(s) for the failure(s) in the latter hatches isn't clear.

Several serials of fowl pox vaccine were examined for extraneous virus, and bacterial and fungal contamination. Several types of bacteria, some fungi, Newcastle virus, and Mycoplasma gallisepticum were isolated from these serials.

Studies on the egg and hatchery transmission of Mycoplasma meleagridis were conducted. The egg transmission of this serotype increased up to the fifth hatch. Following the ninth hatch, the egg transmission began to decrease. Lateral transmission at the time of hatching was observed.

(St. Paul, Minnesota) (ADP a5-21)

In cooperative research at the University of Wisconsin Agricultural Experiment Station the following work was accomplished:

Determination of host parasite relationships - Nature of disease .

Since the time of the last annual report (June 30, 1965) the 11th, 12th, and 13th consecutive turkey flocks have been raised in the Meteoropathology Building.

The 11th flock of 2500 Broad white turkeys was divided into 2 groups of different bird densities ($1\frac{1}{2}$ sq. ft. and 1 sq. ft. of floor space/bird) and an intentional infection with Mycoplasma gallisepticum was initiated in both groups at 7 weeks of age. The birds were then followed to market age with frequent bird sampling for necropsy examination, Mycoplasma isolation attempts, and serological studies. Crowding was determined to have an adverse effect as evidenced by a higher incidence of air sac lesions, and M. gallisepticum recoveries, a higher mortality rate, and condemnation rate at time of slaughter.

Aerosol stability studies were conducted with Mycoplasma gallisepticum and Mycoplasma meleagridis. From the results of these studies it appears that viable Mycoplasma may survive in the aerosol state for at least 36 hours and possibly longer.

Aerosol infection studies with Mycoplasma gallisepticum indicated that chickens exposed to low aerosol concentrations may develop an HI antibody response before they develop an agglutinating antibody that can be detected on the routine plate test. Many birds with positive HI antibody titers never became positive on the plate test.

Studies on isolation and characterization of the causative agents resulted in the following:

Mycoplasma meleagridis was more resistant to drying than M. gallisepticum, remaining viable on filter paper at 4° C for 4 to 6 weeks compared to less than 1 week.

Antigen production with M. meleagridis was improved by introducing small amounts of inoculum of organisms in the log phase of growth into a pre-warmed medium, agitating the culture, and harvesting when the viable count was near the highest value.

A further modification of the French Medium resulted in a formulation that is easily prepared, and supports rapid growth of all avian Mycoplasma.

A complement fixation test has been developed for identification of avian Mycoplasma isolates.

Preliminary studies indicated that a quantitative complement fixation test can be used for accurate assay of growth of antigen strains.

Preliminary studies also indicated that the complement fixation test could be used to detect specific antigens in tissues.
(Madison, Wisconsin) (ADP a5-21)

Public Law 480 grant to the Hebrew University, Hadassah Medical School, Jerusalem, Israel, initiated research on "the effects of prolonged feeding of theraphthalic acid (TPA) to rats." This work is being done in connection with Food and Drug Administration requirements that TPA be proved noncarcinogenic before it can be used as a potentiator of some antibiotics in attempts to control respiratory disease (principally Mycoplasma infection) in poultry.

At this time the facility for the specific pathogen-free (SPF) animals has been completed and the trial operations completed with respect to sterilization, food preparation, personnel instruction, etc. SPF rats of 2 different strains were obtained from the Radiobiological Institute of the Organization for Health Research, TNO Rijswijk Z.H., the Netherlands, and breeding has started. The rate of production of litters seems to be satisfactory and there is every likelihood within the next half-year that the various groups will be started for the definitive assay of theraphthalic acid (TPA). (Jerusalem, Israel) (AlO-ADP-8)

B. Salmonellosis.

At the Southeast Poultry Research Laboratory, Athens, Georgia, further selection has been made of Salmonella typhimurium cultures for preparation of rapid whole-blood and serum plate agglutination antigens. Five cultures with well developed somatic and flagellar antigenic factors have been used to prepare experimental lots of rapid plate and tube agglutination antigens. These antigens are presently under evaluation using whole-blood and serum from S. typhimurium-infected adult and semiadult chickens. Six different agar mediums have been investigated for both bacterial growth yield at different pH values and antigenic sensitivity. A medium having as its basic ingredients trypticase-soy-cysteine-glycerine (TSCG medium) yielded, in general, more growth per strain than did any of the other 5 mediums studied. Neotetrazolium chloride has been successfully used to vitally stain S. typhimurium cells both on solid mediums and in harvested suspensions. A repository for Salmonella cultures of avian origin has been established at the Southeast Poultry Research Laboratory.

Methods for studying Salmonella penetration of the three areas of the outer structures of the chicken egg have been further perfected and refined. It is now possible to sample penetration of microorganisms under the shell,

between the membranes, and on the inner surface of the inner membrane of the same egg in less than 2 minutes. It has been demonstrated that both motile and nonmotile Salmonella can readily penetrate to the internal substance of the egg. Penetration patterns of Salmonella organisms have been related to the temperature and humidity of incubation and to various physical and chemical treatments to which eggs are exposed prior to penetration studies. The effects of formaldehyde fumigation and a variety of chemical dusts in altering the potential penetration of Salmonella organisms through the egg surface have been determined. It has been demonstrated that formaldehyde rapidly leaves the egg surface after fumigation and does not penetrate into the egg during the fumigation process.
(Athens, Georgia) (ADP a5-30)

C. Pasteurellosis.

At the National Animal Disease Laboratory, Ames, Iowa, a chemically defined medium was developed that supports good growth of Pasteurella multocida. This medium will aid in determining the minimal nutritional requirements and the nature and synthesis of cellular constituents, which should aid in defining the pathogenesis of fowl cholera.

Previous work has shown that precipitates obtained from 2 strains of Past. multocida by centrifugation of saline extracts of the cells are toxic, immunogenic, and serologically specific. Further studies have shown that they are physically and serologically heterogeneous. Attempts to separate the mixture by column chromatography with 2% agarose resulted in high losses. Some separation was obtained by density gradient centrifugation in sucrose. Two main fractions, No. 40 and No. 50, were obtained by high speed centrifugation. Both fractions were toxic in rabbits and chicks, but No. 40 killed at lower doses. Intravenous injection of 1 or 5 µg. of No. 40 actively immunized chicks, but No. 50 did not. Analysis of fraction No. 40 showed it to contain both heptose and galactose, whereas No. 50 was free of heptose and contained galactose. The double diffusion patterns of both fractions against rabbit antisera significantly different. These findings will aid in obtaining a purified immunogenic antigen that can be chemically defined. (Ames, Iowa) (ADP a7-25)

D. Newcastle Disease.

In basic research on Newcastle disease at the National Animal Disease Laboratory, Ames, Iowa, the characteristic structure and morphology of stable, intact Newcastle disease antigen was established by electron microscopy. Controlled degradation of the intact antigen and purification of its parts was then studied in preparation for investigations of the chemical and immunogenic nature of its structural subunits. Knowledge of

the subunits is necessary for acquiring high quality antigen both for efficient vaccines and serological applications. Chemical analysis of the subunits correlated with their immunogenicity would be mandatory in accumulating knowledge useful for the production of synthetic antigen.
(Ames, Iowa) (ADP a5-28)

At the Southeast Poultry Research Laboratory, Athens, Georgia, an apparatus has been constructed for the exposure of chickens to predictable, measured concentrations of airborne microorganisms. This technique of aerosol exposure has the advantage of closely paralleling natural exposure in that it does not artifically circumvent any of the natural body defenses of the bird.

The aerosol apparatus was used for challenge exposure of chickens of different genetic backgrounds with Newcastle disease virus in cooperation with personnel from the Animal Husbandry Research Division. Preliminary results indicate that it will be possible to develop from a heterogenous flock, strains of chickens with increased susceptibility and strains with increased resistance to Newcastle disease virus.

The procedures for growing cell cultures from the kidneys of 4- to 7-week-old chickens were established. The cells are being used to explore the interference phenomenon between infectious bronchitis virus and Newcastle disease virus with the hope of elucidating the mechanisms involved. These 2 viruses are commonly used simultaneously in commercial vaccine preparations. (Athens, Georgia) (ADP a5-28)

In cooperative research at the University of Maine, the specific pathogen-free (SPF) program in broilers and breeders has made it possible to study the epizootiology of several virus diseases of poultry on over 7 million birds in 400 flocks since 1961. This has encouraged the poultry industry to begin positive efforts to eradicate Mycoplasma gallisepticum. During 1965-66 over 500,000 birds were serologically tested for this disease.

The use of killed virus Newcastle disease vaccine is still recommended as the most logical and safest method for disease prevention in broiler production in the specific pathogen-free program.
(Orono, Maine) (ADP a5-28)

In cooperative Newcastle disease research at the Wisconsin Agricultural Experiment Station, Madison, the mutation rate of the red plaque was consistent with that of other viruses, as opposed to published reports. A striking selective influence of host was found upon the growth and survival of identifiable plaque-type clones in natural heterogenous populations. This was characterized by successive dominance of certain plaque-clones in tracheal and cloacal exudates as well as in the elimination of some plaque-clones in

certain host systems. Study of characters associated with virulence of pure clones and heterogenous strains for chickens was continued. In one heterogenous population, a large clear plaque was a million times more virulent than a small clear plaque. The third component, a red plaque, was still less virulent as only irregularly was it lethal for chickens even when 10^9 infective units were administered.

Evidence of prolonged survival of ND virus of identifiable plaque types was obtained in decomposing carcasses and in defined substrates of known pH.

Markers for lentogenic strains were identified. Acid stability and affinity for horse erythrocytes and chicken brain cells distinguish B1, La Sota, and F strains. Strains isolated from vaccine breaks and contaminated non-Newcastle disease virus vaccines were both similar to and different from recognized lentogenic strains.

The repository continues to supply virus and occasionally reference serum to qualified investigators in the United States and abroad. In 1966, 27 strains were sent to 11 institutions. (Madison, Wisconsin) (ADP a5-28)

Under a PL 480 grant to the Veterinary Research Institute, Pulawy, Poland, research on Newcastle disease has produced the following results:

The unsatisfactory results of liquidation of Newcastle disease virus (NDV) in nature by using mass vaccination could not be ascribed to the changes of field strains coming in contact with the partially immunized population. Because of the gradual rise of immunologic differences between the changing field strain and the stable strains used for vaccine production, the latter can assure immunity satisfactory to prevent the development of the disease but insufficient to prevent the inapparent infection of birds. This creates the reservoirs of the virus.

To imitate the variation phenomena under the influence of antibodies, selective passages of NDV in the presence of immune serum are used.

Twenty selective passages of mesogenic Roakin and lentogenic La Sota strains were carried out in the presence of immune serum in chick embryo cell cultures. The derivative strains obtained by this procedure were tested for immunogenic properties by estimation of HI antibody level in vaccinated hens. These values were lower than those after vaccination with the original strains. However, the HI level is not always reliable proof of the degree of immunity. For instance, Provost et al. found in hens vaccinated with a NDV strain grown in cell cultures, a marked immunity in spite of a very low titer of HI antibodies. On the basis of Bankowski and Corstvet's suggestions it may be assumed that in such cases the

antibodies contained in respiratory, reproductive, and nervous systems play a more important role than those circulating in serum.
(Pulawy, Poland) (E21-ADP-6)

Under PL 480 grant to the Veterinary Research Institute, Pulawy, Poland, work has continued on "environmental stress as a contributory factor in animal diseases."

To supplement the investigations made in the previous years on stress in poultry, one additional experiment was made on 77 chickens. The administration of large quantities of ascorbic acid before the infection and after the infection had no therapeutic effect on the infection with Newcastle disease virus. For comparative purposes, the preparation, Influmin (Flumidin, Flumadon, Virugon), which is supposed to exert some therapeutic properties with human influenza, has also remained without any positive effect.

Also, experiments were conducted with swine to study the effects of fasting, truck transportation, 39° C heat and cold of 2° C below zero on the behavior of biochemical indices in blood, such as glucose, ascorbic acid, cholesterol, cholinesterase, Na, K, Ca, Mg, P, and Cl. By means of plastic markers, investigations were also made on the effects of transportation by truck on food passage rate in pigs. Most of these experiments also studied the influence of psychotropic drugs, chlorpromazine, dimenhydrinate, and amphetamine on control swine and those under stress.

The accomplished experiments have proved that (as previously shown on chickens) fasting, transportation, heat, and cold stress factors do contribute in the evolution of biochemical changes in the organisms of swine. Transportation stress diminished significantly the rate of food passage through the alimentary tract of pigs and that the psychotropic drugs had some moderating influence on the stressed animals. About 6000 biochemical determinations were carried out in this work.
(Pulawy, Poland) (E21-ADP-7)

E. Infectious Bronchitis.

At the Southeast Poultry Research Laboratory, Athens, Georgia, a Georgia field strain has been passaged in tissue cultures of embryonic chick lung. This virus causes a moderate destruction of the tissue cultures, and retains its infectivity for birds. It has changed somewhat in its antigenic potential.

Studies with IBV have shown that this virus is more resistant to the effects of heat when it is in a solution of $MgSO_4$, Na_2HPO_4, and Na_2SO_4. Salts such as NaCl, KCl, $MgCl_2$ failed to show this effect. The virus was particularly

unstable in water alone. There was 10,000 to 100,000 times more virus in solution of effective salts than in water after 10 minutes at 50° C. This may be important in the administration of water vaccines. It does tend to support the grouping of IBV as a myxovirus. A serum neutralization test procedure has been adapted to IBV. Results with this test indicate that it can be used to show antigenic differences between strains of the previously described types. It has been used to demonstrate differences and similarities within the Massachusetts, Connecticut, and Iowa types, and to identify one strain as an apparently new type.

(Athens, Georgia) (ADP a5-23)

At the National Animal Disease Laboratory, Ames, Iowa, a cell culture plaque reduction method of detecting infectious bronchitis virus neutralizing antibodies was evaluated and compared to the standard method using the embryonating chicken egg. The cell culture plaque reduction method was more advantageous to use since it requires 2 days rather than 7 days to complete. It is less subject to nonspecific neutralization and is not subject to serum dilution factors as is the embryonating egg system.

Fluorescent antibody studies showed that infectious bronchitis virus only replicates in the epithelial cells of chicken embryo kidney, liver, lung, and air sac cell cultures. The liver cells do not show fluorescence until 6 hours as compared to 4 in cells from the other organs.

Fluorescence in lung cells differs from other cell types in that they do not show diffuse fluorescence but only a granular type. This study points out the fact that one reason for one type of cell culture being less sensitive than another is the relative number of susceptible cells in the culture and also the number of cells that can be initially infected.

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AREA NO. 6 - INFECTIOUS AND NONINFECTIOUS DISEASES OF FUR ANIMALS

Problem. In the raising of fur animals in captivity, such as mink, rabbits, and foxes, disease problems incidental to the confinement of such animals are encountered. These include viral, bacterial, parasitic, mycotic, nutritional, and hereditary diseases. Virus diseases of mink cause the greatest loss to the 5,000 mink ranchers now producing more than 7 million pelts annually valued in excess of $130 million. The role of helminths as carriers of rickettsial and viral agents causing, or associated with diseases of fur animals, is becoming extremely important and one about which little is known.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving microbiologists and veterinarians engaged in both basic studies and the application of known principles of the solution of infectious and noninfectious diseases of fur animals. Research was conducted on the following diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 3.1 scientific man-years. This effort was applied as follows:

Coordinated Field and Laboratory Studies 1.1 at the U. S. Fur Animal Disease Research Laboratory, Pullman, Washington, in cooperation with the Washington State University, and under a cooperative agreement on encephalopathy of mink with the Wisconsin Agricultural Experiment Station, Madison.

Transmission of Infectious Diseases by Helminths 2.0 at the Endoparasite Vector Pioneering Research Laboratory, Pullman, Washington, in cooperation with the Washington State University.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 4.3 scientific man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Field and Laboratory Study of the Diseases of Fur Animals.

Isolation of serum fractions capable of producing Aleutian disease in mink - Serum protein fractionation of pooled mink serum containing the Aleutian disease (AD) principle was used in an attempt to achieve some relative purification of the agent capable of producing the disease in mink. All

fractions of zone electrophoretic separations produced the disease when injected into normal mink.

Some relative purification of serum protein fractions capable of producing AD was achieved using gradient elution DEAE (diethylaminoethyl)-column chromatography.

The influence of Aleutian disease infection in the dam on reproduction - It was found that the Aleutian disease virus reduced the number of live kits at birth. Moreover, it was determined that Aleutian mink were more severely affected than non-Aleutian mink.

Use of Pathogenic Feline Panleukopenia Virus (FLV) to Immunize Mink Against Mink Virus Enteritis - A high dose of pathogenic feline panleukopenia virus given subcutaneously to mink interferred with mink virus enteritis given orally. Mink were protected even when the vaccine was injected 8 hours after the mink virus enteritis challenge inoculation.

Serum Proteins in Mink and Cattle Affected with the Chediak-Higashi Syndrome (C-HS) - This study indicates that increased susceptibility of C-HS animals to chronic infections is related to abnormal granules (lysosomes). These granules occur in various granule-producing cells throughout the body, including the leukocytes, and the condition is not a result of impaired ability to produce gamma globulin.

Tests for Distemper Virus Neutralizing Antibody - With the conventional fertile egg test, antibody was demonstrable in ferrets 8 days following a single subcutaneous injection of virus while probit analysis of data from the single dilution test revealed the appearance of antibody as early as 4 days post-immunization.

The thermostability of distemper virus was studied at various temperatures. It was concluded that 16 hours at 25° C provided an adequate and convenient incubation for the virus antibody reaction in a sensitive test for distemper virus neutralizing antibody. (Pullman, Washington)(ADP a6-7)

A Transmissible Scrapie-Like Disease of Mink - In cooperative work at the Wisconsin Agricultural Experiment Station, the virus of mink encephalopathy has been serially transmitted in mink. The disease has been characterized by a long incubation period of 9 to 12 months and degenerative lesions in the brain. The causative virus has been shown to have unusual stability. All these properties are characteristic of scrapie virus of sheep.

There has been no evidence of vertical transmission of this virus in mink and very little evidence of horizontal transmission. The mink have been

readily infected by feeding them diseased tissues. Presumably, infected meat was the source of the naturally-occurring disease in Wisconsin mink.

The incubation was reduced from 12 months following ingestion of virus to 4½ months by intracerebral passage. The central nervous system lesions include astrocytosis, and vacuolization of the neurons and extraneuronal brain substance. The susceptibility of mice, ferrets, cattle, and monkeys is being studied. There is preliminary evidence that mice and ferrets can be infected. Tissue culture studies have been disappointing, although explants from the brain of infected mink produce more outgrowth of astrocytes than do explants from the brain of normal mink. This outgrowth has also been from explants from the brain of scrapie-infected animals.

(Madison, Wisconsin) (ADP a6-7)

B. Persistence and Transmission of Viral and Rickettsial Diseases in Helminths.

At the present time this pioneering project is in the construction phase. Scouting trials will be conducted in an attempt to learn something about the viral flora of certain parasites of ruminants. Isolations will be attempted from collected and submitted specimens.

(Pullman, Washington)(ADP a3-10)

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AREA NO. 7 - MISCELLANEOUS INFECTIOUS AND NONINFECTIOUS DISEASES OF ANIMALS

Problem. Included in this area of research are studies on problems involving more than one species of domestic animal, poisoning by various plants, which differ in toxicity according to local conditions, and affect different species of animals in various ways; agricultural chemicals such as herbicides and pesticides, which may produce poisoning in animals, especially if not properly used, and may also leave dangerous residues in the soil, feed, or animal body, and bloat, a common, serious condition in cattle and sheep. Investigations of these diverse problems require modern techniques as well as fundamental approaches through chemistry, pathology, physics, physiology, and other scientific disciplines. The problems are so complex, diverse, and numerous that it has been impossible to more than scratch the surface in probing for basic knowledge required for protection of the nation's livestock and poultry populations.

USDA AND COOPERATIVE PROGRAM

The Department has a continuous long-term program involving biochemists, microbiologists, pathologists, physicists, and veterinarians engaged in both basic studies and the application of known principles to the solution of miscellaneous infectious and noninfectious diseases of animals. Research is being conducted at the designated locations.

The Federal scientific effort devoted to research in this area totals 24.2 scientific man-years. This effort is divided among sub-headings as follows:

Components of Normal and Immune Serum 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Preparedness for Diagnosis of Foreign Animal Diseases 0.5 at the Plum Island Animal Disease Laboratory, Greenport, Long Island, New York.

Alleviators and Diagnostic Tests for Plant Poisoning 1.0 Poisonous Plants Laboratory, Logan, Utah.

Biochemical Effects of Agricultural Chemicals 1.0 at the ADP Field Station, Toxicology Laboratory, Kerrville, Texas, and through a cooperative agreement with the Stephen F. Austin College at Nacogdoches, Texas.

Detoxication Mechanisms in Cattle and Sheep 1.0 at the ADP Field Station, Toxicology Laboratory, Kerrville, Texas.

Cytological Responses to Antiparasitic and Other Agricultural Chemicals 1.0 at the ADP Field Station, Toxicology Laboratory, Kerrville, Texas.

Toxicological and Pathological Effects of Pesticides 1.2 at the ADP Field Station, Toxicology Laboratory, Kerrville, Texas and through a cooperative agreement with the Texas Agricultural Experiment Station, College Station, Texas.

Mycotic Diseases of Domestic Animals 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Proteins and Other Complex Molecules from Animal Disease Agents Derived Primarily from Surface Structures and Extracellular Products 1.5 at the National Animal Disease Laboratory, Ames, Iowa.

Chemical and Physical Studies on Microbial Antigens 1.5 at the National Animal Disease Laboratory, Ames, Iowa.

Microbiology of the Ruminant Digestive Tract and Its Relation to Digestive Disturbances 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Physiology of Normal Mammalian Cells Grown in Tissue Cultures 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Metabolic, Antigenic, and Pathogenic Characteristics of Dermatophilus congolensis 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Delineation of Motor Centers in the Brain that are Associated with Motility of the Ruminant Esophagus and Stomach 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Physiological Fate of Rumen Gases Absorbed from the Lungs Following Eructation 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Correlation of the Ultrastructural and Biological Properties of Animal Pathogens 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

The Effects of Mycotoxins on Animals 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Relationship between Psittacosis-group Agents Found in Wild and Domestic Birds and Domestic Mammals 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Teratogenic and Toxic Compounds from Poison Plants 1.0 at the Poisonous Plants Laboratory, Logan, Utah.

The Role of Parathyroid Hormone and Thyrocalcitonin in Calcium Metabolism 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Studies of Pituitary-adrenal Function in Cattle 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

The Toxicological Effects of Loco Plants on Livestock 0.5 at the Poisonous Plants Laboratory, Logan, Utah.

Development and Modification of Equipment for Greater Laboratory and Animal Room Safety 0.5 at the National Animal Disease Laboratory, Ames, Iowa.

The Role of Physical, Chemical, and Biological Aerosols in Domestic Animal Diseases 0.5 at the National Animal Disease Laboratory, Ames, Iowa.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 25.8 scientific man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Components of Normal and Immune Serums.

At the National Animal Disease Laboratory, characterizations of high and low molecular weight homologous and heterologous antibodies in rabbit serum following exposure to virulent Brucella abortus have been continued and extended to serums obtained after exposure to virulent Brucella melitensis. The progress report of these studies appears under line project ADP a1-32. (Ames, Iowa) (ADP a7-14(Rev.))

B. Preparedness for Diagnosis of Foreign Animal Diseases.

An additional facility has been completed to accomplish laboratory assistance in diagnosis. During the fiscal year, the existing facilities were utilized to develop data. The results were accepted as a basis for safe introduction into the United States of biologicals necessary to implement research in human medicine. Results of tests conducted in the facilities were used as a basis for the importation into the United States of semen from Charolais cattle and for the importation of a virus from Australia subsequently submitted to a state university for characterization studies. Assistance was provided to representatives of the Animal Health Division in the differentiation and identification of suspect vesicular lesion material from Mexico. Progress was continued in the collection and development of biological materials and were added to the biological reference center. (Greenport, New York) (ADP a7-16)

C. Develop Alleviators for Poisonous Plants.

Larkspur, Delphinium species, is one of the most serious poisonous plant problems to the cattle industry. A mineral supplement has been formulated and put on a range with a history of a high incidence of larkspur poisoning to see if the incidence couldn't be decreased or losses prevented altogether. No losses have occurred thus far this year. Evaluations for the prevention of range losses can only be made after several years of study because of the large number of variable factors present. (Logan, Utah) (ADP a7-17(Rev.))

D. Biochemical Effects of Agricultural Chemicals and Control Substances.

Research continued in developing knowledge about the normal enzymes of cattle and sheep; this year aldolase, creatine phosphokinase and glutamic oxaloacetic transaminase were studied. Serum proteins, albumin and 3 globulins, were studied by electrophoresis and by special buffer separation.

Young diary calves were depleted of tocopherols by special feeding, then dosed with demeton or coumaphos to determine any increased susceptibility. No increase was noted. Results in commercial practice indicate there is a relationship between certain poisonings and Vitamin E (tocopherols). This year, however, we did not duplicate the conditions essential to produce poisoning. (Kerrville, Texas) (ADP a7-18)

In cooperative research with the Stephen F. Austin College, an expression relating the change that occurs in the velocity of sound in air when aerosol particles are introduced has been derived from more realistic assumptions than those previously used. The changes in the velocity are dependent upon the frequency of the sound and the distribution by size of the aerosol particles. A size distribution has been determined from experimental data on the changes in the sound velocity. This distribution agrees to a considerable extent with the distribution obtained through laborious photographic and counting techniques. The need for further refinements to the experimental procedures is indicated. Electromagnetic scattering by aerosol particles is being considered as a basis for developing a particle size spectrometer. Spectrographic studies of the glow from an ionovac speaker have been made.

In other cooperative research at the College on preparation and characterization of barium antimony tartrates a constant temperature bath was constructed with a sensitivity within 0.1° C. The bath was equipped to handle low temperatures as well as above-room temperature. A stirring apparatus was constructed to be used in conjunction with the bath for the solubility study. Analytical procedures were worked out for all ions present in the solution. Efforts to locate the temperatures, at which barium antimony tartrate was formed, proved unsuccessful. Both dilatometric and conductivity methods were tried. (Nacogdoches, Texas) (ADP a7-18)

E. Detoxication Mechanisms in Cattle and Sheep.

Oximes. The oximes 2-PAM, P2S, and TMB_4, considered to be reactivators of cholinesterase, were studied for the blood levels attained and maintained after intravenous injection. As expected, maximum levels were attained at, or just after, completion of the injections. Unexpectedly, the levels attained were far less than should theoretically be the case, reaching only 5 to 10% of theoretical. Each oxime disappears rapidly from the blood, TMB_4 less than the others. In 6 to 10 hours the oximes have essentially disappeared from the blood. Poisoning by coumaphos did not appear to influence the blood level or disappearance of 2-PAM.

2,4,5-T. The propylene glycol butyl ether ester of 2,4,5-T was rapidly hydrolyzed by raw meat samples from sheep to the parent 2,4,5-T acid. Methods specific for this ester would miss the converted 2,4,5-T. Studies were made on the excretion of the ester and of 2,4,5-T acid and on the residues in tissues after a variety of dosages. From single doses, 86% could be recovered in urine as unchanged ester, and 1.6% as 2,4,5-T acid. In blood a peak concentration of 100 p.p.m. was reached 3 hours after a 25 mg./kg. dose. From 5 hours post-dosing onward, 2,4,5-T was recovered in the blood in less than 1 p.p.m. amounts. At 7 days post-dosing the ester could not be recovered from the tissues. The 2,4,5-T was found at less than 0.005 in liver and 0.013 in urine. Sheep were fed 5 or 25 p.p.m. of ester of their diets for 4 days. At the lower level the ester predominated in the urine; at the higher level 2,4,5-T acid was predominant. In blood, both ester and acid were recovered at both levels; the ester was predominant.

Bromophos. Sheep were dipped 9 times at weekly intervals in 0.5% bromophos emulsions. At 1, 8, and 22 days after the final dipping, omental fat samples were obtained. At 1 day, the fat contained from 5 to 15 p.p.m. of bromophos, at 8 days 1 to 2.5, at 22 days 0.07 to 0.43 p.p.m.
(Kerrville, Texas) (ADP a7-19)

F. Characterization of Cytological Responses to Toxic Actions of Pesticides and Other Agricultural Chemicals in Livestock and Poultry.

The chemosterilants tepa, metepa, apholate and HEMPA were administered daily to birds to develop tissues for microscopic examination. Because of absence of the project leader (for graduate training) limited research has been done in this project this year. (Kerrville, Texas) (ADP a7-20)

G. Toxicological and Pathological Effects of Insecticides, Herbicides, Fungicides, and other Agricultural Chemicals on Livestock and Poultry.

Abate. Abate shows considerable promise as a mosquito larvicide and, in studies by USPHS of laboratory animals and man, a very satisfactory margin of safety. In sheep and cattle the low toxicity seemed also true. It has

been administered in a variety of ways. Although studies are still current, it is safe to say both these classes of livestock can tolerate at least 20 times the amount of Abate that would be present in water during control operations.

Sodium Fluoride. Certain researchers have advocated sodium fluoride as an antidote for organophosphorus compound poisoning, based on studies in mice. Attempts to use the compound in sheep failed to yield satisfactory results.

Potentiation. To determine if phenothiazine drenches would potentiate the effects of parathion as they do coumaphos, sheep were drenched once a week for 3 weeks, then dipped in parathion suspension (0.025%, previously toxic). No poisoning resulted. Potentiation certainly did not occur.

Insecticides Used on Crops. Parathion, demeton, carbophenothion, and phosdrin have been studied to determine their hazard to animals grazing treated forage. Demeton was objectionable to sheep in some way. Sheep would not eat treated forage unless absolutely starved to it.

Herbicides. Twenty-seven commonly used herbicides were studied to determine minimum toxic tosages for cattle, sheep, and chickens, or all 3 species.

The results confirm our earlier conclusions that most of the commonly used organic herbicides are not hazardous to livestock and poultry when used as recommended. In most cases, even moderate carelessness would not result in poisoning.

Insecticides. Sixty-one insecticides were studied during the fiscal year in cattle and sheep to determine acute and subacute toxicity.
(Kerrville, Texas) (ADP a7-23)

Through a cooperative agreement with the Texas Agricultural Experiment Station tissue sections representing 79 experimental animals treated with 12 pesticide compounds by the Animal Disease and Parasite Research Division, Kerrville, Texas, have been examined for pathologic changes. The compounds include herbicides, fungicides, and insecticides. While the studies are of a preliminary rather than definitive nature, they serve to indicate important changes in tissues associated with poisoning and secondary sequelae and to indicate the similarities in the changes produced by compounds of similar composition. (College Station, Texas) (ADP a7-23)

H. Mycotic Diseases of Domestic Animals.

The antigens present in the culture filtrates of N. asteroides have been studied serologically by gel precipitin and complement fixation techniques. The gel precipitin technique has been used to demonstrate serologic similarities and differences among strains of N. asteroides and other members of the genus.

Because not all serologic relationships are demonstrated with one technique, the study has been extended to include use of antigen-sensitized erythrocyte techniques. (Ames, Iowa) (ADP a7-24)

I. Chemical and Physical Studies on Microbial Antigens.

Previous work has shown that precipitates obtained from two strains of Pasteurella multocida, by centrifugation of saline extracts of the cells for 2 hours at 105,000 x g are toxic, immunogenic, and serologically specific. Further studies have shown that they are physically and serologically heterogeneous. Attempts to separate the mixture by column chromatography with 2% agarose resulted in high losses. Some separation was obtained by density gradient centrifugation in sucrose. However, better separations were obtained with a series of high speed centrifugations. Two main fractions were obtained. The first, No. 40, corresponded to the precipitate obtained after 1 hour at 105,000 x g; and the second, No. 50, to the precipitate obtained at 165,000 x g from the 105,000 x g supernatant. Both fractions were toxic in rabbits and chicks, but No. 40 killed at lower doses. Intravenous injection of 1 or 5 ug. of No. 40 actively immunized chicks, but No. 50 did not. Analysis of fraction No. 40 showed it to contain both heptose and galactose. However, No. 50 was free of heptose and contained galactose. The double diffusion patterns of both fractions in rabbit antisera were significantly different. These findings will aid in obtaining a purified immunogenic antigen that can be chemically defined. This research was carried out cooperatively with Line Project ADP a7-25, Investigation of the genus Pasteurella, which is assigned to the section on Bacterial and Mycotic Investigations. (Ames, Iowa) (ADP a7-29)

J. Microbiology of the Ruminant Digestive Tract and Its Relation to Digestive Disturbances.

Pure cultures of important ruminal bacteria were examined for their ability to synthesize the essential amino acids phenylalanine, leucine, valine, and tryptophan from corresponding acids with one less carbon than the amino acid. The biosynthetic reactions involved are important both to the host and to the microbes and are different from any that have been demonstrated in other organisms. Most of the rumen bacteria tested (8 of 11 species) use the new pathway involving phenylacetate for synthesis of phenylalanine. At least three species, Bacteroides ruminicola, Ruminococcus flavefaciens, and Peptostreptococcus elsdenii, use the carboxylation pathway to synthesize valine and leucine carbon skeletons from isobutyrate and isovalerate, respectively.

Labeled tryptophan was synthesized when the mixed microbial population from either cattle or sheep was incubated with indole-3-acetic acid-1-C^{14}, but only one pure culture (Ruminococcus albus) has been found that is able to conduct this synthesis. Since the acid precursors of these amino acids are

produced in the rumen from the same amino acids, the series of reactions constitute an amino acid cycle that has not been previously described. The mechanism of the reductive carboxylation reactions that must occur is not known. (Ames, Iowa) (ADP a7-30)

K. Metabolic, Antigenic and Pathogenic Characteristics of Dermatophilus congolensis.

Dermatophilus congolensis is the causative agent of cutaneous streptothricosis in cattle, horses, goats, game species and man. The means of transmission of the causative agent of this disease is not clear. Ticks and flies have been suggested as being involved in the transmission of the zoospores of this organism and also of a closely related species, D. dermatonomus.

Dermatophilus congolensis was transmitted from infected to normal rabbits by stable flies, Stomoxys calcitrans, and by house flies, Musca domestica. Mechanical disruption of the host's skin by the feeding fly was not necessary for transmission. Moistening of both the lesions on donor rabbits and fly feeding sites on recipient rabbits enhanced fly transmission. Stomoxys calcitrans transmitted the infection for periods up to 24 hours after feeding on an infected rabbit. Dermatophilus congolensis was demonstrated on the feet of S. calcitrans and was isolated from M. domestica.
(Ames, Iowa) (ADP a7-32)

L. Delineation of Motor Centers in the Brain that are Associated with Motility of the Ruminant Esophagus and Stomach.

Electronic equipment has been developed to study behavior patterns of domestic animals. This development will be useful in studying subclinical physiopathological changes in toxin and disease studies.
(Ames, Iowa) (ADP a7-33)

M. Physiological Fate of Rumen Gases Absorbed From the Lungs Following Eructation.

Using carbon-labeled CO_2 and CH_4 it was demonstrated that considerable quantities of these two gases were absorbed from the lungs following eructation. Appreciable amounts of $C^{14}H_4$ are absorbed and oxidized. Following intravascular introduction of $C^{14}H_4$, radioactivity has been demonstrated principally in simple carbohydrates of liver tissue of sheep. This is the first work showing that mammalian tissue can oxidize methane.
(Ames, Iowa) (ADP a7-34)

N. Correlation of the Ultrastructural and Biological Properties of Animal Pathogens.

An investigation was conducted to establish the identity of an agent

isolated from a field case of mucosal disease in North Dakota. Techniques of tissue culture cytopathology, immunofluorescence, and electron microscopy were utilized. A Herpes-like characteristic particle was readily recognized by electron microscopy in infected tissue cultures. This agent rapidly outgrew the agent of mucosal disease in tissue culture. The immunofluorescent studies confirmed identification of this Herpes-like virus as infectious bovine rhinotracheitis virus.

In other investigations, mild extraction of Pasteurella multocida yielded antigenic substances. The derived antigens were shown to be membranous sacs of relatively small size by electron microscopy, i.e., less than 0.1 micron in diameter. In the fowl immunization trials, these particulate antigens were highly effective in conferring protection against challenge exposure with virulent organisms. The chemical and biological properties of these particulate antigens suggested that they may be related to the endotoxic components of P. multocida.

A study was made on the anatomical features of Vibrio fetus and the general ultrastructural details of the organism's cell wall, cytoplasm, nucleoplasm, and flagella. New anatomical findings were that (1) the cytoplasmic membrane was found to consist of a complex honeycomb-like matrix similar to structures found in Spirillum and Rhodospirillum microorganisms; (2) cytoplasmic inclusions, previously referred to as "polyphosphate granules," were bounded by a single membrane; and (3) the flagella had a cone-shaped basal granule associated with the cytoplasmic membrane. It was also noted that the flagella of Vibrio fetus are enlarged over a short region near the site of attachment to their basal granule. This enlarged portion was more resistant to mechanical shear than the remainder of the flagellum.

A report presenting the details of optimized conditions for the isolation of some major antigenic components of various strains of Vibrio fetus was prepared. Cell wall material and the flagella were partially characterized by biophysical and biochemical methods. Electron microscopy was used to monitor the progress of this study. All physical techniques tested for the preparation of cell wall material yielded substantial amounts of the complex cytoplasmic membrane associated closely with the cell wall. Flagella were readily removed from the cell by short-term treatment in a Mickle shaking machine with sand (3 min.). The results of several chemical and enzymatic treatments of crude cell wall materials suggested that the morphology of the cell was not determined simply by a single structural element but was more likely the result of the interaction of the several membranous components of the cell's envelope.

(Ames, Iowa) (ADP a7-35)

O. Relationship Between Psittacosis-group Agents Found in Wild and Domestic Birds and Domestic Mammals.

It has been observed that large doses of a highly virulent, avian isolate

of a turkey psittacosis agent (Chlamydia), which were lethal for many laboratory animals, failed to affect pigeons and sparrows. It was theorized that the unusually high normal body temperature ($43^{\circ}C \pm 0.5^{\circ}$) of pigeons and sparrows might be a factor in the resistance of these birds to this strain. Therefore, the in vitro rates of inactivation at 43° C of the organisms' infectivity for chicken embryos and the toxicity for mice were determined in comparison with an isolate of a strain normally found in pigeons and which caused disease in pigeons and sparrows. Growth rates of both isolates at 43° C in cultures of embryonic chicken tissues were also compared.

The turkey isolate was inactivated in vitro at a consistently rapid rate at 43° C while the pigeon isolate was relatively resistant to 43° C inactivation. In another test, the toxin or mouse lethality factor possessed by the turkey isolate was slowly inactivated at 43° C, but the toxin of the pigeon isolate that had been heated for 24 hours at 43° C remained infectious for both pigeons and sparrows. However, the turkey isolate failed to affect either species whether or not the isolate was heated. These results suggested that because of its 43° C sensitivity, the turkey isolate was less able than the pigeon isolate to survive and cause disease in birds whose normal temperature was 43° C.

After a study of the various attempts over the past 36 years to classify the organisms of the psittacosis-lymphogranuloma venereum-trachoma group, the project leader has proposed that all of these organisms be united taxonomically and nomenclaturally under a single genus, Chlamydia, Jones, Rake and Stearns 1945, with the type species being C. trachomatis (Busacca) Rake 1957. The taxonomic reasons for unifying the agents of the PLT group under a single genus were that all of the organisms had a common morphology, common developmental cycle, and common group antigen. These factors have greater taxonomic importance than the more variable factors of pathogenicity, or specific serology. Furthermore, because of their chemical, physiological and morphological similarity to bacteria, the terms "virus," "large virus," or "rickettsial agent" were incorrect and misleading when applied to the chlamydiae. They can properly be called chlamydiae bacteria, or "procaryots," a term meaning "primitive nucleus." A Taxonomy Subcommittee of the American Society for Microbiology concurred in 1966.

(Ames, Iowa) (ADP a7-37)

P. Teratogenic and Toxic Compounds from Poisonous Plants.

The study of plants poisonous to livestock is important because ingestion of such plants by livestock under grazing conditions is very common. These plants may cause severe physiologic effects including death in the animal that ingests the plant or else fetal deaths, abortions, or malformations in the offspring from pregnant animals. The laboratory is investigating these effects in animals ingesting members of the Veratrum, Lupine,

Astragalus, and Oxytropis genera. The objective is to identify the active agent from the plants by extraction of the plant material, preparation of fractions and pure compounds and then characterization of the active material. Certain alkaloids from Veratrum appear to be the active compounds, while in the other named plants studies have not progressed beyond preparation of active mixed fractions. As the active compounds become identified, we can then study the molecular mechanism of action. We can then suggest and test the efficacy of remedial action for the deleterious effects produced by these poisonous plants. (Logan, Utah) (ADP a7-38)

Q. Role of the Parathyroid Hormone and Thyrocalcitonin in Calcium Metabolism.

The bovine thyrocalcitonin has been isolated and characterized. It was similar, but not identical, to the porcine thyrocalcitonin. Young pigs have 3 to 4 times the plasma level of thyrocalcitonin as the older pigs.

The role of the thyroid gland in relation to calcium has been studied in the pig and plays an important protective role in certain conditions of increased levels of plasma calcium. (Ames, Iowa) (ADP a7-39)

R. Studies of Pituitary-Adrenal Function in Cattle.

Surgical procedures have been developed to permit collection of adrenal blood from conscious calves. Analytical techniques have been worked out to quantitate corticosteroid concentrations in bovine adrenal venous blood. These techniques have been applied to measure corticosteroid secretory rates in normal Holstein calves. (Ames, Iowa) (ADP a7-40)

S. Toxicological Effects of Loco Plants on Livestock.

Astragalus lentiginosis and Oxytropis sericea were fed to bred ewes from the 10th to the 30th day of gestation and the 25th to the 45th day of gestation. Both plants produced fetal abnormalities during both periods fed. The abnormalities observed were a flexure of the knees, lateral rotation of the front legs and a looseness in the stifle and hock joints. These abnormalities have been observed under field conditions.
(Logan, Utah) (ADP a7-41)

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AREA NO. 8 - FOOT-AND-MOUTH AND OTHER EXOTIC INFECTIOUS DISEASES OF CATTLE

Problem. The Congress in 1948 authorized establishment of a laboratory in the United States for research on foot-and-mouth (FMD) and other exotic animal diseases. The law required that the laboratory and related facilities for research and study be located on a coastal island separated from the mainland by deep, navigable waters. Plum Island was selected as the site for the laboratory on July 28, 1952. The Plum Island Animal Disease Laboratory as a U. S. Department of Agriculture venture came into existence on July 1, 1954, and since that time this laboratory has been responsible for protecting the nation's livestock industry against animal diseases of foreign origin. Foot-and-mouth disease has visited the United States on 9 occasions and each time has been eradicated. The last outbreak of FMD was in 1929. Contagious bovine pleuropneumonia was eradicated in the 1880's and has not recurred since. Success in keeping these exotic animal diseases out of the United States has been due to a number of factors and a continuing vigilance by U. S. Department of Agriculture personnel.

The establishment of the Plum Island Animal Disease Laboratory and its continuing research program on exotic animal diseases has provided a laboratory in the United States where research on animal disease foreign to our soils is carried out. As new information is developed at the laboratory, it is made available to those agencies in the Department responsible for keeping out livestock animal diseases which do not occur in this country. Foot-and-mouth disease is capable of reducing our overall productivity by 25 percent in areas where it might become established. The disease exists in all large land areas of the world with the exception of Central and North America, Australia, and New Zealand.

Rinderpest, a disease of cattle, continues to be a serious disease problem in Africa and Asia. This disease is capable of killing 90 percent or more of the cattle exposed to it. Other diseases for which the laboratory is responsible include contagious bovine pleuropneumonia, Rift Valley fever, East Coast fever, and lumpy skin disease. All of these diseases continue to cause severe losses in other parts of the world. The possibilities of entry of these diseases in the United States continues, primarily because of the progressively increasing scope, speed, and extent of modern international transportation. Information developed at the Plum Island Animal Disease Laboratory is applied to the protection of the nation's livestock against foreign animal diseases.

The research continues to develop and maintain a competence for diagnosis of exotic animal diseases. Fundamental research is being conducted on biological, chemical, and physical properties of the infective agents that may be useful in prevention, control, and eradication of these diseases.

USDA AND COOPERATIVE PROGRAM

The Department at its Plum Island Animal Disease Laboratory has a continuing long-term program involving veterinarians, biochemists, biophysicists, microbiologists, and pathologists engaged in basic and applied research in this problem area. All of this research is conducted at the Plum Island Animal Disease Laboratory, Greenport, New York, except for supplemental field studies on FMD vaccines that are conducted cooperatively in The Netherlands. The Department is also engaged in research under terms of an Interagency Agreement with the Agency for International Development, U. S. State Department, in Kenya, on contagious bovine pleuropneumonia.

The Federal scientific effort devoted to research in this area conducted solely at the Plum Island Animal Disease Laboratory, totals 23.5 scientific man-years. This effort is divided among sub-headings as follows:

Studies on Foot-and-Mouth Disease Virus 2.5.

Determine Mechanism of Antibody Formation 1.0.

Quantity Production of Foot-and-Mouth Disease Virus 2.0.

Establishment and Characterization of Cell Lines and Cell Strains 1.0.

Mechanism of the Interaction Between Foot-and-Mouth Disease Virus Molecules and Host Cells 2.0.

Genetic Biochemistry of Foot-and-Mouth Disease Virus 1.0.

Effects of Chemical and Physical Environment on Foot-and-Mouth Disease Virus 1.5.

Bulk Freeze Drying of Foot-and-Mouth Disease Virus Vaccine and Antiserum 1.0.

Identification, Purification, and Chemical and Physical Characterization of Foot-and-Mouth Disease Virus and Other Exotic Animal Viruses 2.0.

Immuno-chemical Investigations of Foot-and-Mouth Disease Virus 1.5.

Attenuation of Representative Types of Foot-and-Mouth Disease Virus 1.5.

Biological Mechanism of Natural Resistance and Susceptibility to Foot-and-Mouth Disease Virus 1.5.

Biological Alteration of Foot-and-Mouth Disease Virus from Continual Residence in Cell Cultures 1.0.

Morphological Aspects of Virus-Cell Relationships 1.0.

Diagnostic and Immunizing Procedures for Contagious Bovine Pleuropneumonia 3.0.

Work was continued under a PL 480 grant to the Instituto Biologica, Sao Paulo, Brazil, for a 5-year study of tissue culture of indigenous strains of foot-and-mouth disease virus (FMDV), and experimental field vaccination.

Under a PL 480 grant to the Ministry of Agriculture, Laboratories of Foot-and-Mouth Disease and Tissue Culture, Etlik, Turkey, research continues on "studies of various indigenous types of FMDV, and the production of a vaccine for the control of foot-and-mouth disease in Turkey."

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Studies of Foot-and-Mouth Disease Vaccine.

Cattle and swine vaccinated with completely inactivated FMDV vaccine containing mineral oil adjuvant had a high degree of protection when exposed to infection between the 3rd and 14th day after vaccination. Some 95 percent of cattle and 80 percent of swine had no signs of the disease when exposed to infected animals. Resistance was not closely related to serum-neutralizing antibody and was not significantly influenced by the day of exposure to virulent virus. These studies show that vaccination could be applied close to a focus of infection with expected good protection.

Of particular interest was the observation that the immune response, as shown by antibody levels, was not influenced at all by the exposure to the virulent virus.

The virus used in the vaccine was purified and measured to a known weight of antigen per dose of vaccine.

The immune response obtained in cattle inoculated with the treated inoculums used in swine was similar to the response obtained in swine. Steers inoculated with chemically-treated baby hamster kidney (BHK) cell culture virus and the oil adjuvant were resistant to infection when exposed to FMD-infected steers, whereas, steers inoculated with the treated material combined with aluminum hydroxide gel were not. Statistical analysis of the mouse PD_{50} values of the serums indicated a significant difference between the groups inoculated with treated material and oil adjuvant and those inoculated with the treated material and aluminum hydroxide gel.

(Greenport, New York) (ADP a8-8(Rev.))

B. The Mechanism of Antibody Formation (Studies on the Effects of Glycidaldehyde on Foot-and-Mouth Disease Virus (FMDV)).

Suspensions of FMDV A119 propagated in BHK cells and bovine kidney (BK) cells were treated with 0.02% GDA for 4 hours at 37° C and tested for infectious virus in BK cell cultures, suckling mice, and steers. Residual infectious virus was detected in steers, while no virus could be found in BK cells and suckling mice using small volumes of treated virus suspensions. However, infectious virus could be detected in the latter test systems when large volumes of treated virus were tested.

Infectious and treated virus preparations were antigenically potent and produced antibody levels protecting animals against high challenge doses of infectious virus. The mean PD_{50} values for groups of guinea pigs inoculated with a 1:25 and a 1:125 dilution of nontreated antigen were 1.98 and 1.82, respectively, while undiluted 1:5, 1:25, 1:125 dilutions of GDA-treated virus produced mean PD_{50} values of 1.56, 0.90, 0.42, and 0.01, respectively.
(Greenport, New York) (ADP a8-10(Rev.))

C. Quantity Production of FMDV

Studies were continued toward the development of basic information on virus-cell interrelationships that would be applicable to better methods for detection, assay, and production of virus in cells grown on glass and in suspension.

Methods have been developed for preparation of primary cultures of bovine calf kidney cells with higher susceptibility to infection with strains of the 7 types of FMDV, especially with virus directly from the animal.

For 19 FMD viruses (16 cattle strains representing all 7 types) cell cultures were about equal with cattle in sensitivity for virus detection for 10 viruses, superior to cattle for 9, superior to mice for 11, and about equal with mice for 8 viruses. Cattle were superior to mice for 5 viruses, and mice were superior to cattle for 6 viruses. The other 8 viruses were detected at similar dosage levels by cattle and mice, but the percentage of takes was generally higher in cattle. Four of the 6 viruses, for which the 3 test systems were about equal in sensitivity, were type O. Guinea pigs were much less sensitive to the 19 viruses than were the 3 other test systems.

The approximate minimum infective dose, expressed in bovine ID_{50} units of a cell culture-adapted virus of strain A119, was 0.5 unit in cattle, 0.0001 in mice and 0.00002 in cell cultures.

Primary cultures of bovine kidney cells prepared by the method developed in Cytological Investigations supported the formation of plaques under agar

overlay of all strains of FMDV (representing the 7 types) available at PIADL as well as all the FMDV modified by residence in chronically infected cells. Primary cultures of bovine kidney cells prepared by conventional methods did not support the formation of plaques under agar overlay of some of the viruses directly from cattle tongue or some of the viruses modified by residence in chronically infected cells.

Strains of the 7 types of FMDV were grown in liter amounts in 5-liter Povitzky bottle cultures of bovine kidney cells. The virus inoculum came directly from cattle tongue. The mean concentration of virus was $10^{8.2}$ PFU per milliliter; the average yield per cell was 317 PFU.

(Greenport, New York) (ADP a8-12 (Rev.))

D. Establishment and Characterization of Cell Lines and Cell Strains.

Plaque formation titers under agar overlay of strains of the 7 types of FMDV of bovine origin in a line of BHK cells were inferior to plaque formation of these viruses in primary cultures of bovine and swine kidney cells.

Cell lines of porcine kidney and embryonal bovine tracheal mucosa were susceptible to strains representing the 7 types of FMDV.

(Greenport, New York) (ADP a8-14 (Rev.))

E. Mechanism of the Interaction of FMDV Molecules and of Other Exotic Viruses with Host Cells.

Electron Microscopy of FMDV Development. Electron micrographs of thin sections of pig kidney (PK) tissue culture cells heavily infected with FMDV show crystalline arrays of virus in the cell cytoplasm. The arrays occurred in cells 5 hours after infection and were in different states of development. The crystals were generally in regions of cytoplasmic vacuolization, areas with altered mitochondria and moderately osmophilic "grey bodies." In one test, approximately 1 out of 30 PK cells examined contained virus crystals.

Effect of FMDV Infection on Host Cell Deoxyribonucleic Acid (DNA), Ribonucleic Acid (RNA) and Protein Synthesis. The BHK-21 cells were infected with a high multiplicity of FMDV, type A119. Under these conditions, 80 to 90 percent of the tissue culture cells were infected. The FMDV infection inhibits host cell DNA and protein synthesis almost completely 5 hours post-inoculation. However, RNA synthesis is inhibited only 45 to 55 percent. This finding required the use of Actinomycin-D to study FMDV RNA replication. The inhibition of host cell protein synthesis is being used in the laboratory as a means of measuring the extent of FMDV infection in BHK-21 cells.

Electron Microscopy of African Swine Fever Virus (ASFV). An electron microscopy study was made of the hemadsorption reaction between ASFV-infected swine leukocytes and red cells. Normal leukocytes did not absorb

red cells, while moderately infected ones showed specific attachment. Virus replication sites in leukocytes were visible, but virus emerged only occasionally. Virus exterior to the leukocyte did not appear to be necessary for successful hemadsorption.

Interaction of 2 ASFV isolates, Lisbon '57 and Tengani, with ferritin-conjugated antibodies to Lisbon '57, Tengani, Salamanca and Hinde isolates was studied by electron microscopy. Considerable cross reaction was found among the 4 isolates. Cross reactions were similar in complement fixation and agar gel diffusion precipitin tests. The particular virus isolates used for cell infection resulted in generally stronger reactions with Tengani than with Lisbon '57. Although the host cell material, acquired as another layer on ASFV during cell emergence, does not appear to entirely negate the virus-antibody reaction, the ability to distinguish between isolates is uncertain and virus neutralization does not occur.

(Greenport, New York) (ADP a8-17)

F. Genetic Biochemistry of FMDV.

Control Mechanisms in FMDV-Infected Cells. Studies on the mechanism of FMDV-induced repression of host cell transcription and translation have included possible changes in histone control, and the effects on in vitro nuclear chromatin-transcriptase (aggregate enzyme) activity. In vivo methylation of protein and nucleic acid and in vivo acetylation of protein-infected cells have also been investigated. Kinetic studies of total histone following virus infection by pulse labeling with ^{14}C lysine correlated closely with the decrease in total protein synthesis. Nuclear aggregate enzyme preparations from BHK cells were isolated at 90, 180, and 300 minutes after infection. The in vitro decreased rate of incorporation of ^{3}H uridine triphosphate into acid insoluble products paralleled the decrease of host cell RNA synthesis observed in vivo. Isolation of cytoplasmic soluble, nuclear histone, and nuclear acid-insoluble fractions, after in vivo pulse labeling with ^{3}H or ^{14}C acetate, revealed that viral-induced inhibition of protein synthesis did not inhibit protein acetylation. Increases in acetylation found in the cytoplasm, where FMDV replicates, may be initiated by virus. Preliminary studies on pulse methylation of host-cell RNA using ^{3}H-labeled methylmethionine showed a 20 to 30 percent greater inhibition of methylation of ribosomal and soluble RNA than for incorporation of labeled uridine into the same RNA fractions.

Ribosomes and Polyribosomes in BHK Cells. The role of ribosomes on host cell protein synthesis during FMDV replication is being investigated. Studies have as yet been carried out only on uninfected BHK-21 cells. Intact cells were labeled with isotopic amino acids for short time intervals (5 to 10 minutes) and then lysed. Cells were incubated in a maintenance medium with bovine serum and lysed by several methods considered optimal for polyribosome stability. The soluble proteins, ribosomes, and polyribosomes

were resolved in linear sucrose gradients. No polyribosomes were detected, and all labeled amino acids were found as soluble protein, on monomeric ribosomes, and in some cases on a membrane fraction. Two major difficulties were encountered: (1) Active ribosomes were attached to cell membranes requiring the use of detergents for their release. This treatment may have caused dissociation of ribosomes from messenger RNA and/or release of ribonuclease (RNase) from lysosomes. (2) The BHK-21 cell is fragile and easily lysed by mechanical manipulation. This prevents the repeated washing of cells required to remove RNase contributed by the serum. Results indicative of a low level of polyribosomes were obtained by incubating cells in mediums without serum. In addition, the cell sheet was rinsed several times with isotonic buffer following incubation with labeled amino acids.

FMDV RNA Replication in Cells. The RNA was extracted from BHK-21 cells infected with type A119 FMDV in the presence of Actinomycin-D. Virus-specific RNA components, resolved by ultracentrifugation in sucrose gradients, were first detected between 90 and 180 minutes after infection; the maximum rate of synthesis was found 5 hours after infection. The virus-specific RNA components, which have been identified, are viral RNA and a complex having a sedimentation coefficient of about 20 S which is partially ribonuclease resistant. The RNase-resistant RNA is synthesized in amounts similar to viral RNA, and "pulse labeling" experiments indicate that it is formed before virus RNA.

FMDV-Specific Replicase Activity. Mengo-virus infected L cells and polio-virus infected HeLa cells possess an enzyme, replicase, not found in uninfected cells. This enzyme, an RNA polymerase, is essential for reproduction of virus-specific RNA, and there is strong evidence that it is coded for the infecting viral RNA molecule.

Baby hamster kidney cells infected with FMDV were examined for the presence of a viral-induced replicase. Crude enzyme preparations were represented by suspensions of infected cellular mitochondrial-microsomal pellets in sucrose-magnesium solutions at protein concentrations of roughly 5 mg/ml. The enzyme assay system measured the incorporation of tritiated uridine triphosphate into an acid-insoluble product. The assay medium contained a high energy phosphate bond generating system, magnesium, Actinomycin-D, and the 4 nucleotide triphosphates required for RNA formation. Infected BHK cells had significant levels of replicase activity. Activity was dependent on the concentration of magnesium, but strongly inhibited by manganese. This indicated that the enzyme preparations were not contaminated by DNA-dependent RNA polymerase found in normal cell nuclei. In addition, Actinomycin-D, which interferes with the transcription of DNA information into RNA molecules, did not affect the enzymatic activity. Each of the individual nucleotide triphosphates was required for maximal enzymatic action.

The reaction rate was linear for the first 10 minutes of incubation at 37°C and reached completion at 60 minutes, an approximate 2.5-fold greater incorporation of isotope than at 10 minutes. The acid-insoluble product was hydrolyzed by both ribonuclease and 0.3 N sodium hydroxide, indicating its polyribonucleotide nature.

Replicase activity in heavily infected cells was maximal at about 4 hours and decreased after 5 hours. This correlates with the known maximal appearance of viral infectivity at about 5 hours and the reported instability of replicase. At low multiplicities of infection, harvests taken at 18 to 24 hours contained little replicase activity, probably because of thermal instability of the enzyme.

Viral RNA replication was studied in the cell-free system by using enzyme preparations obtained from infected cells treated with Actinomycin-D and pulse labeled briefly (1 to 30 minutes) with ^{14}C uridine. The crude enzyme preparations contain, in addition to replicase, the RNase-resistant RNA, and newly synthesized viral RNA. The 20 S RNase-resistant RNA and 37 S viral RNA appear to be synthesized in the cell-free system.

(Greenport, New York) (ADP a8-18)

G. Effects of Certain Chemical and Physical Treatments on FMDV.

A number of surface-active agents (29), chosen because of structural differences representing the various kinds of such chemicals, were investigated for ability to inactivate FMDV. None were found in concentrations up to 5 percent which could inactivate the virus at 28°C in 30 minutes through their chemical effect alone. However, one compound was capable of viral destruction, but only by reason of the pH level engendered by it in the mixture. It is possible to remove a contaminating virus from a valuable antiserum by chemical means without too great a loss of specific antibody. Beta propiolactone in a concentration of 0.3 percent was capable of destroying a contaminating FMDV from an antiserum of a different type of FMDV. However, complement-fixing antibody activity was lost with no observable change in neutralizing antibody titer. With physical means, filtration of such a contaminated antiserum through a 10 mμ APO membrane resulted in removal of the contaminating virus, no loss of complement-fixing antibody and a neutralization index loss of 1.6 logs. Some loss of antibody is experienced, therefore, by either chemical or physical methods of decontaminating an antiserum. Acceptable antigens have been prepared using acetylethyleneimine (AEI) at the 0.05 percent level when mixed with FMDV at 23°C and reacted together for 24 hours as a crude preparation or for 12 hours if the virus has been clarified by centrifugation. A similar preparation of virus was exposed as a 1-mm. film at 4°C to 48 microwatts of energy from an ultraviolet source for 10 minutes. Following treatment with 0.05 percent concentration of BPL at 37°C for 15 minutes, it was inactivated and was as antigenic in laboratory animals

as the antigen prepared with AEI. There is a good probability that it may be possible to separate bovine-adapted FMD virus from that adapted to tissue culture. This is because of their separate reaction to divalent salts such as $MgCl_2$ in either 1 or 2 M concentration at 37 and 50°C. The bovine-adapted virus is preserved over a period of days as compared to tissue culture-adapted virus of the same strain which is immediately degraded under the same conditions. The simple chemical, hydroxylamine, has the ability to inactivate FMDV. The antigenicity of such inactive preparations was best demonstrated when a concentration of 0.25 M was added to virus and the mixture reacted at either 4 or 23°C. This reaction takes from 6 to 18 hours to inactivate the virus. Adult chickens and mice inoculated with inactive preparations of virus from such treatment produced a good titer of neutralizing antibodies.

(Greenport, New York) (ADP a8-19)

H. Bulk Freeze-Drying of FMDV, Vaccines, and Antiserum.

It is possible to freeze-dry and store FMDV at 4°C in flame-sealed ampules with expectation of little or no detectable loss for at least 2 years. This has been accomplished by attempting to define the conditions of freezing, drying, and storage which are most conducive to preservation of the virus. This study has been helped by the fact that FMDV is quite resistant to changes in its environment which could be deleterious to the stability of other viruses such as rabies or measles. The problems encountered with rubber sealed containers are the loss of vacuum and entrance of moisture through the interstices of the rubber diaphragm closures. This may be solved by silicone-impregnated butyl rubber closures.

(Greenport, New York) (ADP a8-20(Rev.))

I. Identification, Purification, and Chemical and Physical Characterization of FMDV.

Methods of Extraction of Infectious RNA from Pure FMDV and Its Analysis by Moving Zone Ultracentrifugation in Linear Sucrose Gradients. The RNA was extracted from pure FMDV by two general procedures. One involved phenol extraction either alone or with such additions as detergent or ethylenediaminetetraacetic acid (EDTA). The other procedure involved virus degradation at pH 5 and RNA extraction with sodium dodecylsulfate (SDS). The extracted RNA was centrifuged through a linear sucrose gradient, and the contents were flow monitored at 260 mμ in a Gilford Model 2000 recording spectrophotometer. Appropriate fractions were then analyzed for infectious RNA by a highly sensitive assay. Infectivity banded in the region of the major optical density peak with sedimentation coefficient of about 37 S. The SDS-extracted RNA fractions yielded better RNA profiles and were usually more infectious than RNA extracted with phenol. The best profiles had 75 percent of the optical density in the 37 S region.

Polypeptides in FMDV Protein. The coat protein of FMDV, purified and solubilized from phenol or from whole virus preparations, has been treated with several reagents to minimize inter- and intra-molecular bonding to determine the number of peptides. Electrophoretic examination of the protein under sieving conditions revealed several components. Eliminating sieving conditions caused all components to increase in mobility so that only a single mobility zone was observed. Since this zone was present even under sieving conditions, current investigations are directed toward resolving the slower, sieved components as aggregates of the faster component.

Electrophoresis of FMDV on Cellulose Acetate Strips. Electrophoretic analyses on cellulose acetate strips of FMDV, type A, strain 119, with different passage histories revealed differences in homogeneity and response to chymotrypsin-induced mobility changes. Virus with high-passage history in calf kidney (CK) cells migrated as a single zone at the pH and ionic strengths examined. This virus, purified after a single additional passage in BHK cells, derived from clone 13, also gave a single zone. In contrast, virus purified after a single passage in uncloned BHK cells revealed 2 electrophoretic zones as determined by staining and infectivity. The mobility of one zone corresponded to that of the single zone obtained from the high-passage CK virus. All low-passage virus had only a single electrophoretic zone.

After incubation with chymotrypsin, differences between FMDV with low and high passage histories were indicated by the increase in mobility of only high-passage virus. Changes in mobility induced by chymotrypsin were not detected by sedimentation studies in the analytical ultracentrifuge. Only the mobility of the faster moving zone of the heterogeneous virus was changed by the enzyme. Trypsin, ribonuclease, and diisopropylfluorophosphate-inactivated chymotrypsin had no effect.

Analysis of purified FMDV over a range of pH and ionic strength conditions revealed a downward shift in isoelectric point with increasing chloride ion concentration. Isoelectric point data and ionic strengths were used to calculate the Longsworth and Jacobsen equation constants needed for determining the isoelectric point of FMDV at any ionic strength. The virus was always inactivated at or near its isoelectric point. It was stable, however, at pH 5 at high ionic strength or pH 9.5 at low ionic strength. Such pH values are separated from the isoelectric point by at least 2 pH units.

Optical and Biological Measurements During Zone Electrophoresis in a Glucose Gradient. Highly purified, concentrated FMDV, type A, was homogeneous and monodisperse by carrier-free zone electrophoresis in a glucose density gradient. Virus mobilities in veronal-acetate buffer, pH 8.6 and ionic strength 0.1, were identical whether determined by infectivity or optical

methods. Low passage CK virus had a significantly higher mobility, 3.28×10^{-5} cm^2/volt.sec., than high passage virus, 2.84×10^{-5} cm^2/volt.sec. Single passages of the high passage virus in BHK cells did not change its mobility. Regardless of passage history, storage at 4^oC in 0.05 M phosphate at pH 7.5 increased mobilities to about 3.75×10^{-5} cm^2/volt.sec. with retention of infectivity; some virions broke down to protein and RNA. These electrophoretically distinct viruses possessed different absorbance-temperature profiles, but identical sedimentation constants.

Application of Digital Computers to Ultracentrifugation. A detailed program for processing moving boundary ultracentrifuge runs on a 32K digital computer was completed. It incorporates the following concepts: (1) Quadratic least squares analysis provides the initial s-rate, the s-rate internal to the data and a measure of its constancy. (2) The time of intersection of peaks in multicomponent runs permits determination of the true starting time. (3) Interpolating polynomials permit baseline calculation not optically visible in the region of a peak. (4) Appropriate summation of possibly irregularly spaced ordinates of the schlieren peak permits calculation of the concentration for each frame. (5) Internal standard errors of all quantities can be used to assess reliability and to reveal aberrant data. (6) Line-printer plots of boundary movement and apparent distribution function permit rapid interpretation of computer results. (7) Tests and decisions by the computer during execution are useful before resubmitting the converted data for more sophisticated analyses.

The program is useful for double cell runs with up to 4 components in each for both conventional and synthetic boundary cell experiments during sedimentation or flotation.

Gamma Irradiation of FMDV. Gamma irradiation of crude and pure FMDV reported last year has been extended to FMDV RNA. In addition, the rates of inactivation of RNA extracted from irradiated virus and of intact FMDV were found to be essentially identical. The size of the infectious moiety of FMDV will be calculated from the results obtained. Changes in absorbance-temperature profiles of irradiated FMDV and FMDV RNA correlated reasonably well with loss of infectiousness induced by the irradiation.

Ultrasensitive Assay for Infectious RNA of FMDV. A method has been devised for improving the detection of infectious FMDV RNA by 3 to 7 orders of magnitude. In some trials, the biological detection of RNA was 100 to 1000 times more sensitive than the detection of the virus from which the RNA was derived. This brought the RNA plaque count to within 1/10 of the electron microscope virion count versus a ratio of about 1/1000 for virus plaque forming units per virions.

In addition, the method succeeded in demonstrating infectious RNA for transmissible gastroenteritis virus.

Cell Cultures and Pure FMDV in Quantity. A tissue culture cell and virus production unit is now in operation. Forty mg. of pure FMDV is produced each week in this unit.

(Greenport, New York) (ADP a8-25)

J. Immuno-Chemical Investigations of FMD.

A new component has been found and generally characterized that is produced in tissue cultures or animals infected with FMDV. This material is distinct from the recognized virus particles and their protein subunits. It has been tentatively termed the "virus infection-associated" (VIA) antigen, because it does not appear to be a constituent of the virus, but is produced as a consequence of the infectious process. The VIA antigen occurs only in FMD infection, but it appears to be immunologically the same regardless of the serological type of FMDV that induced its formation. For this reason, it may be responsible for many of the difficulties encountered in the diagnostic typing of this disease agent.

Immunological techniques have been developed that permit a reliable estimate of the weight of FMDV in crude preparations. This procedure provides a rapid and simple assay technique for the standardization of vaccines and for the evaluation of materials obtained during purification studies of the virus. The preservative, Merthiolate, had a deleterious effect on both virus particles and VIA antigen. This finding has explained certain incongruous results obtained in earlier investigations, and has provided information that may be of value in studies on the physical and chemical nature of the virus.

(Greenport, New York) (ADP a8-26)

K. Attenuation of Representative Types of FMDV.

A newly isolated FMDV, type O, strain 9, was grown on CK cells after 1 passage in PIADL cattle. The virus was exposed to ultraviolet light to induce variation. It was also treated with cold phenol to extract infectious RNA. The RNA was further treated with nitrous acid to induce variation. The survivors and progeny of these stresses were plated on calf kidney cells and isolates selected for further study. A total of 8 isolates and the parent strain were obtained and to these were applied a battery of simple tests to detect differences between isolates after stressing and the parent strain. Major changes were not found. Minor changes or differences were found but these always occurred with a small plaque variant, which may have been a minor portion of the parent virus population.

(Greenport, New York) (ADP a8-27)

L. The Survival of FMDV in Meat and Meat By-Products.

Even though gross skin lesions of FMD in infected cattle are usually confined to the feet and sometimes the teats, the virus may be found in nearly all areas of the normal appearing skin. The virus may persist in the skin for as long as 5 days after cessation of viremia. Thus, hides taken from FMD-infected cattle may be potentially dangerous from a disease control standpoint. Four conventional hide preservation methods were tested and found ineffective for inactivation of FMDV: (1) Green-salting with storage at either 4° or 15°C (virus found up to 352 and 90 days, respectively). (2) Brining in saturated sodium chloride solution for 20 hours and storage at 15°C (virus found up to about 28 days). (3) Drying at 20°C and 40% relative humidity (RH) (virus found for as long as 42 days). (4) Salting for 7 days followed by drying at 20°C and 40% RH (virus found up to 21 days).

Detection of trace amounts of FMDV in tissues and fluids of infected animals and infectivity of the viruses for cattle are fundamental parts of this project. Thus, it was essential to determine the limitations of the various test systems for the virus strains studied. For 18 FMDV strains, the approximate minimum infective dose (MID), expressed in bovine ID50 units, varied from 0.01 to 2.0 units for the tongue route of inoculation in cattle, from 0.01 to 6.0 units for inoculation of mice, and from 0.002 to 0.3 units in calf kidney cell cultures. The tongue route of inoculation of cattle was from 100 to 200,000 times more sensitive than the intramuscular route. The viruses that produced the larger plaques on cell cultures were the more infectious, as demonstrated by intramuscular inoculation.

(Greenport, New York) (ADP a8-28)

M. Studies on the Biological Mechanisms of Natural Resistance and Susceptibility to FMDV.

Natural resistance is defined as inborn resistance to an infectious agent and is not related to the formation of antibodies. Recently, much effort has been devoted to learning why some animals are naturally resistant to infection or become so at some time during their lives.

The high degree of susceptibility to FMDV of unweaned mice, contrasted with the pronounced resistance as they mature, affords an excellent opportunity for studying natural resistance to this virus. Previous work revealed that greater virus production and earlier virus multiplication occurred in suspensions of cells from young (susceptible) mice than from old (resistant) mice. The present work was concerned with a comparison of the virus-adsorbing ability of cells from resistant and susceptible mice to see if differences existed between the two groups.

In both groups of mice, adsorption occurred with minced kidney, brain, and lung, but not with heart, spleen, liver, thymus, and skeletal muscle. Homogenates of kidney, brain, and lung also adsorbed, but homogenates prepared from the other tissues mentioned failed to do so. More adsorption occurred with homogenates than with minced tissues.

Centrifugation experiments revealed that the agent responsible for adsorption in brain homogenates is different from that found in kidney homogenates. However, neither brain nor kidney homogenates adsorbed all of the inoculated virus. A fraction of the virus population may be resistant to adsorption under these conditions.

These experiments have revealed no differences in adsorption between susceptible and resistant mice. The evidence indicates that ability of cells to adsorb FMDV is not the decisive factor in susceptibility or resistance of mice. Rather susceptibility of cells, as shown by their ability to support virus multiplication, is the important difference.

(Greenport, New York) (ADP a8-29)

N. Biological Alterations of FMDV from Continual Residence in Cell Cultures.

Viruses were kept in chronic residence in primary BK cell cultures as follows: Virus A119 for 37 months, OM11 for 21 months, C_3 Canefa for 12 months, and a turkey variant of type A for 7 months.

Only the A (turkey variant) chronic residence virus was tested during the period of the report. It showed the same pattern in reduction of virulence and retention of pathogenicity as previously reported for A119 virus kept in chronic residence. Further selection of a nonpathogenic immunogenic virus population from this chronic residence virus by single intramuscular passage in cattle (secondary modification or selection) yielded a virus that produced viremia and specific antibody (but no clinical signs) in cattle.

Primary cultures of canine kidney cells were insusceptible to A (turkey variant) virus at first cell passage, but on second cell passage were susceptible in varying degrees. Three lines of dog cell-adapted virus were developed. Loss of pathogenicity occurred rapidly after serial passage (4, 20, and 27 passages respectively). Test animals inoculated with the nonpathogenic virus failed to develop viremia, presumably on account of large numbers of interfering particles in the virus. Secondary modification or selection of a nonpathogenic, immunogenic population by methods other than cattle passage seem to be indicated in work with this virus.

A type A119 virus modified by continued residence in bovine kidney cell cultures and subsequent selection by intramuscular cattle passage proved to be nonpathogenic and immunogenic in sheep.

Rapid intramuscular serial passage of A (turkey variant) virus in sheep (14 passages) resulted in loss of its ability to cause clinical signs (except fever). The virus produced lesions in cattle, but the character of the clinical signs indicated some reduction in pathogenicity for cattle.
(Greenport, New York) (ADP a8-30)

O. Morphologic Aspects of Virus-Cell Relationships.

Use of Fluorescent Antibodies in the Study of the Cytopathology of FMDV. Preliminary studies on the sequential development of FMDV antigen in primary CK cell cultures using the indirect fluorescent antibody technique revealed specific immunofluorescence 150 minutes following infection but not at 100 minutes. No cytopathic effects were detected at these times. Using high multiplicities of infection to obtain maximally infected cells, the technique did not distinguish between types of FMDV. Specific immunofluorescence was also detected, using infected lamb testes cells, but no infected cells were detected in smears of heart, kidney, and pancreas of suckling mice 24 and 48 hours after infection.

Cytogenesis, Survival, and Transformation in Cell Cultures. Studies were continued to determine long-term biological effects on cell cultures (possible transformation) from FMDV infection. Two lines of recovered cells (swine kidney and BK) have been maintained more than 2 years; 3 lines of infected cells (BK) have been maintained more than 1 year.

Studies were made on the cytogenesis (nature and origin) of cells in primary cell cultures made from hamster kidney cortex. By electron microscopic and histochemical methods it was determined that: (a) The cultures developed mainly from small clumps of cells obtained by breaking down the proximal convoluted tubules in the process of trypsinization. (b) The optimal tubular breakdown from trypsinization occurred earlier in kidneys from weanling hamsters (30 minutes maximum) than in those from adults (90-150 minutes). (c) On growth and proliferation of cells from the pieces of proximal convoluted tubules (to form the monolayer cultures), a process of cell dedifferentiation commenced with the first division of the cells. They lost cytoplasm, had fewer mitochondria, lost their positive periodic-acid Schiff granules and contained more irregular nuclei. These changes in morphology resulted in cells resembling fibroblasts but with important differences from connective tissue fibroblasts.
(Greenport, New York) (ADP a8-31)

P. Diagnostic and Immunizing Procedures for Contagious Bovine Pleuropneumonia.

Contagious bovine pleuropneumonia (CBPP) is a problem in several countries today from the standpoints of diagnosis and immunization.

Common factors to both these problem areas are antibodies to the disease agent. Basic information relating class and type of antibody to presently available diagnostic methods has been developed. These studies will be helpful in improving diagnostic methods and in vaccine evaluation studies.

An effective artificial infection method has been developed. This method may be applied not only as a valuable aid in experimental studies but may also be applied as a more precise challenge method for evaluation of vaccines.

(Greenport, New York) (ADP a8-32)

Q. Studies on FMDV (PL 480 Project).

The workers have continued their epizootiological studies on FMD in Sao Paulo, Brazil. Virus samples were received from 67 locations. Of these, 57 were positive by complement fixation. There were 16 type A, 20 type O, 17 type C, and 1 mixed type O and type C virus. From November 1, 1965 to April 30, 1966, they typed 77 additional samples from 58 locations. Of these, 31 samples from 25 locations were isolated in cell cultures and typed by complement fixation. This epizootiological work has been an important phase of the proposed studies.

In addition to the above, a major part of the efforts has been to study the resistance and susceptibility of several kinds of cell cultures and laboratory animals to FMDV. After a number of transfers in vitro, several BK cell cultures became less susceptible to FMDV grown in primary BK cell cultures. This loss in susceptibility was not correlated with a chromosomal change. It is suggested that this change in susceptibility was due to a selection of cell type during continuous cultivation. In contrast to bovine cells becoming resistant with continuous passage, 3 swine kidney cultures, which had been continuously passaged for long periods, have remained susceptible to FMDV.

The researchers have done considerable work on chromosome studies of cell cultures maintained in their laboratory. This work was well done and is useful in the identification of cell cultures as well as the detection of chromosomal changes and the relation of these changes to loss of susceptibility to FMDV.

(Sao Paulo, Brazil) (S3-ADP-2)

R. Studies on Various Indigenous Types of FMDV, and the Production of a Vaccine for the Control of FMD in Turkey (PL 480 Project).

During the past year, considerable effort has been put forth to develop tissue cultures suitable for propagation of FMDV and for use in the production of a tissue culture vaccine. Good progress has been made with the limited facilities available. The workers are now ready to undertake

comparative studies of various vaccines (Frenkel, Schmidt-Waldmann, and tissue culture) in domestic animals. Part of their problem has been to develop a method that will show how well sheep are protected. Sheep do not show secondary lesions comparable to those seen in cattle. For this reason, the workers have undertaken studies to show how secondary infection in sheep can best be demonstrated. On the basis of work to date, it would appear that viremia studies will afford the most information.

Other work has been done on the production of hyperimmune guinea pig serum for serological tests.

In addition, studies were undertaken to determine if European A vaccines would protect animals against type A22 (Near East) FMDV. Field trials suggested that the European type A vaccines were not efficient but a homologous vaccine prepared with type A22 did afford protection.

Future studies are to be conducted to determine the duration of immunity afforded to cattle and sheep by the various vaccines.

(Etlik, Turkey) (A22-ADP-8)

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AREA NO. 9 - FOOT-AND-MOUTH AND OTHER EXOTIC DISEASES OF SWINE

Problem. Foreign diseases, such as foot-and-mouth disease, African swine fever, and Teschen disease, that occur elsewhere in the world, constitute calculable potential threats to the swine industry of the United States. Foot-and-mouth disease is particularly important because the disease frequently occurs primarily in swine from which it spreads to other susceptible species, such as cattle and other ruminants. African swine fever, which until recently was confined to wild and domestic pigs in Africa, has spread to Portugal, Spain, and France. The disease is of special concern because of its resemblance to hog cholera, with which it may be confused. Moreover, mortality from the disease approaches 100 percent, and there is no specific preventive vaccine. Teschen disease, which causes widespread inapparent infections and occasional involvement of the central nervous system, is another of the foreign diseases to be guarded against. A disease indistinguishable from Teschen disease has appeared in England in recent years. Despite all precautions, any of these diseases may occur in the United States, as likely as not through the medium of modern, rapid international transportation. The Plum Island Animal Disease Laboratory is engaged in studies of foreign diseases of swine for the purpose of developing information for increased protection of the Nation's swine industry.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving veterinarians, biochemists, microbiologists, and pathologists, engaged in basic and applied research in this problem area. Research is being conducted on the following diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 3.0 scientific man-years. This effort is divided among sub-headings as follows:

Foot-and-Mouth Disease of Swine 1.0 at the Plum Island Animal Disease Laboratory, Greenport, Long Island, New York.

African Swine Fever 2.0 at the Plum Island Animal Disease Laboratory in cooperation with the East African Veterinary Research Organization, Kikuyu, Kenya, and in connection with a PL-480 grant to the Patronata de Biologia Animal, Ministerio de Agricultura, Madrid, Spain.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Foot-and-Mouth Disease of Swine.

The antibody development in swine experimentally infected with foot-and-mouth disease (FMD) was similar to the pattern produced in cattle and guinea pigs. The antibody produced in the early stages of infection appeared to be a macroglobulin of the 19S class and the later appearing antibody, a γ-globulin of the 7S class.

The electrophoretic mobility of these antibodies indicates that the macroglobulin or "fast" migrating antibody is in the β_2 - or γ_1-globulin region, while the later appearing or "slow" migrating antibody is in the γ_2-globulin region. Antibody was rapidly formed at a level sufficiently high to be demonstrable by the Ouchterlony agar gel diffusion precipitin (AGDP) technique by the 6th or 7th day following inoculation and remained at a demonstrable level throughout the 180-day duration of the experiment. There was considerable variation in the response of individual swine both in quantity and time at which the antibody classes appeared. In some swine, the 7S-class antibody was not detectable before 21 or 28 days postinoculation (DPI), and in some others, the 19S-class antibody was detectable for the 180-day duration of the experiment.

In the swine inoculated with the chemically-treated virus preparations combined with the oil adjuvant, the appearance of antibody was usually later--between the 15th and 28th DPI. The level of antibody was lower than that produced in FMD-recovered swine.

The antibody formed in swine inoculated with the treated virus and Alhydrogel developed in a different pattern, becoming detectable usually within 7 DPI and reaching a peak level between 10 and 14 DPI, quickly disappearing to an almost undetectable level by the 21st DPI. The antibody appeared to be a macroglobulin of the 19S class only; no 7S-class antibody could be detected by the tests applied. Mercaptoethanol treatment of selected serums removed the antibody activity as indicated by virus neutralization assay in suckling mice and the AGDP test.

It seems that the antigenic mass of the chemically-treated inoculum used in these tests was not quantitatively sufficient to stimulate a high-level synthesis of antibody. Only when the effect of the antigenic mass was sufficiently enhanced by adding a suitable adjuvant--in this case, the oil adjuvant--was a satisfactory high level of antibody produced.

(Greenport, New York) (ADP a9-1(Rev.))

B. African Swine Fever.

It was not possible, despite repeated attempts, to obtain definite evidence of replication of African swine fever virus (ASFV) in rabbits, guinea pigs, or hamsters.

Success was achieved in attempts to obtain replication following experimental inoculation of goats. Cells from goats were also useful components of a diagnostic method. These observations are particularly significant as they may offer new and profitable areas for studies relating to diagnosis and vaccine development.

Results of cross-protection studies, which show a difference in only one of 8 ASFV isolates studied, will be used to delineate more precisely the 40 isolates that should be selected for further study in relation to characterization, development of vaccines, and diagnosis.

Further studies failed to show virus neutralizing antibodies in ASFV. Results of preliminary physical-chemical studies with the virus and its soluble antigens will be further developed to assign the virus a proper designation in a classification scheme and to improve detection of carrier animals and vaccines.

Useful data have been developed from studies of the virus using nonhemadsorption, chronic form of the disease, electron microscopy in cells, sensitivity to ethylene oxide, stability of the modified virus, and attenuation and antibody characterization.

(Greenport, New York) (ADP a9-2(Rev.))

Studies on wart hogs from Kenya and Tanzania have shown that even though these animals may harbor ASFV in their tissues, infection of domestic pigs by direct contact is uncommon. An explanation for this lack of contact transmission is that ASFV was generally located in the lymph nodes. The agar diffusion precipitation test continues to perform satisfactorily as a diagnostic test in pigs dying of acute ASFV.

A nonhemadsorbing ASFV was isolated from Uganda and is under study. Early passages of the Uganda isolate contained both hemadsorbing virus and nonhemadsorbing virus.

(Kikuyu, Kenya) (ADP a9-2(Rev.))

Under the terms of a PL 480 grant, research is being conducted at the Servicio de Patologia, Patronata de Biologia Animal, Ministerio de Agricultura, Embajadores, Madrid, Spain, on rapid and accurate diagnostic methods for African swine fever (ASF). USDA scientists, working on ASF in Africa, developed a laboratory test for the diagnosis of ASF, based on the adsorption of red cells on cultures of buffy coat cells. Only those cells that are infected with ASF virus will adsorb red blood cells. The occurrence of ASF in Spain, and the need to conduct diagnosis on suspect samples provided an opportunity to study this method of diagnosis under actual conditions. The Spanish work has shown the test to be specific for ASF. The virus was detected in 9,206 field samples. They have published variously

on their application of the hemadsorption test for diagnosis of ASF. For the most part, these publications have appeared in Spanish veterinary journals and in publication media of the Office of International Epizootics (OIE), Paris, France.

(Madrid, Spain) (E25-ADP-4)

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AREA NO. 10 - FOOT-AND-MOUTH AND OTHER EXOTIC DISEASES OF SHEEP

Problem. For the early detection of any outbreak of foot-and-mouth disease, comprehensive information regarding its effect on all susceptible species is necessary. The effect of foot-and-mouth disease (FMD) on cattle and swine has been, and is being investigated; however, little information is available pertaining to the disease in sheep. Sheep infected with FMD could serve as a source of infection and initiate the spread of the disease. Although primary research emphasis on exotic diseases of sheep at the Plum Island Animal Disease Laboratory is on FMD because of its great economic importance, other exotic diseases of sheep, such as rinderpest, sheep pox, louping ill, Nairobi sheep disease, and Rift Valley fever, are of concern to the Plum Island Laboratory because techniques and materials may be needed for diagnosis, control, and eradication on short notice and unexpectedly. Such diseases, if introduced into this country, could result in high death tolls or cause serious economic losses among susceptible sheep and other livestock. The problem is one of development of basic information applicable to protection of the nation's sheep from foreign animal diseases; development and maintenance of competence in diagnosis of these diseases; and fundamental research on the biological, chemical, and physical properties of the infectious agents that may be useful in prevention, control, and eradication of these diseases.

USDA AND COOPERATIVE PROGRAM

The Department has recently activated a continuing and long-term program involving veterinarians, biochemists, microbiologists, and pathologists, engaged in basic and applied research in some of the problems in this area.

The Federal scientific effort devoted to research in this area totals 1.0 scientific man-year. This effort is divided among sub-headings as follows:

Foot-and-Mouth Disease of Sheep 1.0 at the Plum Island Animal Disease Laboratory, Greenport, Long Island, New York.

Sheep Pox. Public Law 480 funds have been granted to the Turkish Ministry of Agriculture for a study of vaccines against sheep pox prepared from tissue culture propagated virus. The Madras Veterinary College, Madras, India, has also received PL 480 funds to conduct research on an efficient vaccine for protecting sheep against sheep pox. Sheep pox is indigenous in Turkey and India.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Foot-and-Mouth Disease in Sheep.

It is planned to study the response of sheep to a number of the current inactivated vaccines at present under study in cattle and swine. A paper is in preparation reporting on the persistence of antibodies in sheep following experimental infection with foot-and-mouth disease virus.
(Greenport, New York) (ADP all-1)

B. Sheep Pox.

In Turkey studies have continued toward the preparation of a vaccine against sheep pox from tissue culture propagated virus. Sheep pox virus has been passaged 47 times in cultures of monkey kidney cells, 24 times in cultures of sheep kidney cells, 8 times in cultures of sheep testis cells, and 11 times in baby hamster kidney cells. The workers are continuing to passage the virus in baby hamster kidney cells. Virus passaged in baby hamster kidney cells and inoculated into susceptible sheep produces a lesion measuring about 2 to 2.5 cm across at the site of inoculation. This lesion is accompanied by considerable subcutaneous edema, and the animals show a slight thermal response for 2 to 3 days following vaccination. The lesion that develops at the inoculation site disappears in 3 to 4 weeks following vaccination. Animals so vaccinated were given challenge inoculation with a virulent strain of sheep pox virus and a reaction similar to that which developed at the site of inoculation with the tissue culture-passaged virus developed and subsided within 2 weeks following the challenge inoculation. So far, none of the animals vaccinated with the baby hamster kidney cell virus and subsequently given challenge inoculation with the virulent field strain have died. Thus, there is evidence of attenuation of the sheep pox virus for sheep through passage in baby hamster kidney cells. These studies are continuing and offer promise of developing a good living virus vaccine for sheep pox. (Ankara, Turkey) (A22-ADP-6)

In India studies have been continued on the efficacy of different vaccine doses. Two animals showed a severe local reaction when vaccinated with 50 infectious doses of virus vaccine. Seventeen others showed only nodular thickening at the site of inoculation. In addition, of 6 animals inoculated with 75 infectious doses of virus, only one showed severe ulceration. Six other animals receiving 100 doses showed only a mild reaction and nodular thickening at the site of vaccination. All of the above animals when given challenge inoculation three weeks after vaccination with 25,000 doses of sheep pox virus were immune. In addition to the above, field trials were undertaken with gel-adsorbed vaccine. In these trials, 942 sheep were vaccinated with varying doses of vaccine. With few exceptions, there was only moderate reaction at the site of vaccination. These studies are continuing.

Work was undertaken to study the keeping quality of aluminum gel-adsorbed vaccine. These studies are continuing and are important in determining the efficiency and practicability of a vaccine as far as the conditions that must be maintained in the field to insure its potency.

Studies of 6 field specimens showed no strain variation of the sheep pox viruses from different outbreaks. No immunological differences were observed as a result of cross-immunity tests with one of the field isolates. One specimen was infectious for goats and sheep and further studies are in progress with this virus strain.

Studies are continuing on the egg adaptation of sheep pox virus.
(Madras, India) (A7-ADP-5)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

None.

AREA NO. 11 - PARASITES AND PARASITIC DISEASES OF CATTLE

Problem. The cost of parasitic diseases to the cattle industry of the United States is estimated to be in excess of $400 million annually. Disorders caused by parasites are ubiquitous, generally insidious and often overlooked entirely. Diagnosis is difficult and successful treatments for many of these diseases are not available. Moreover, management practices to avoid spread of parasitisms and to control them are often ineffectual. The problem is to develop, through a planned, balanced program of basic and applied research, knowledge for preventing, controlling or eradicating parasitic diseases so as to provide for healthy cattle, insure adequate supplies of parasite-free beef for an expanding population, avoid or minimize economic losses caused by these diseases, and thereby contribute to a more prosperous agriculture and the national economy.

USDA AND COOPERATIVE PROGRAM

The Department has a continuous long-term program involving biochemists, microbiologists, parasitologists, pathologists and veterinarians engaged in both basic and applied studies directed to the development of measures for the solution to the high and extremely costly incidence of parasitism in cattle. Research is being conducted on parasitic diseases at the following designated locations.

The Federal scientific effort devoted to research in this area totals 19.0 scientific man-years. This effort is divided among subheadings as follows:

Effect of Pasture Mixtures and Pasture Management on Control of Internal Parasites 1.5 at the Regional Animal Disease Laboratory, Auburn, Alabama, and through informal cooperation with the Georgia Experiment Station, Experiment, Georgia.

Host-Parasite Relationship of Coccidial Parasites of Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Clinical and Physiological Aspects of Roundworm Parasitism in Cattle, Including Anthelmintic Treatment 1.5 at the University of California, Davis, under a cooperative agreement with the ARS-USDA.

Investigations of Trichomonad Parasites 1.0 at the Animal Disease and Parasite Research Division Regional Animal Disease Laboratory, Logan, Utah, and under a cooperative agreement with the Utah Agricultural Experiment Station, Logan, Utah.

Host-Parasite Relationship of Intestinal Worms, Cooperia spp. in Cattle 1.0 at the Animal Disease and Parasite Research Division, Regional Animal Disease Laboratory, Auburn, Alabama.

Epizootiological and Ecological Investigations of the Internal Parasites of Grazing Cattle 1.5 at the Animal Disease and Parasite Research Division, Beltsville Parasitological Laboratory, Beltsville, Maryland.

Etiology and Immune Response of Cattle to Winter Coccidiosis 1.0 at the Regional Animal Disease Laboratory, Logan, Utah.

Anaplasmosis of Cattle 4.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Interrelationships of Diet and Parasitic Infection in the Production of Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Histochemistry of Gastro-Intestinal Nematodes of Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Parasites of Cattle with emphasis on Stephanofilarial Species 1.0 at the Animal Disease and Parasite Research Division Regional Animal Disease Laboratory, University Park, New Mexico.

Effect of Stocking Rate and Rotational Grazing on Internal Parasitism of Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Environmental Factors Influencing Parasites and Parasitic Diseases of Economical Importance in Ruminants (Cattle, Sheep, and Alpacas) (PL 480 - Peru).

Investigations on Anaplasmosis, Piroplasmosis and Babesiellosis of Cattle are under way through a PL 480 Grant at the School of Veterinary, Montevideo, Uruguay (PL 480 - Uruguay).

Effect of Host Diet on the Bionomics of the Preparasitic Stages of Nematodes in Cattle Feces 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama, and through informal cooperation with the Georgia Experiment Station, Experiment, Georgia.

Effects of Level, Rate, and Period of Exposure to Larvae on the Establishment and Pathogenesis of Gastrointestinal Nematode Parasites of Cattle 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Life History and Host Parasite Relationship of Nematode Parasites 0.5 under the Regional Animal Disease Laboratory, Auburn, Alabama with research conducted at State College, Mississippi.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 12.1 scientific man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Host-Parasite Relationship of Coccidial Parasites of Cattle.

Scientists at the Animal Disease and Parasite Research Division's Regional Animal Disease Laboratory at Auburn, Alabama, reported the following: Five-year-old cultures of Eimeria alabamensis did not produce infections, while four-year-old ones did. Infected intestinal tissues of calves were used for training purposes in electron micrography and the first micrographs were made of an Eimerian, E. alabamensis, located inside the nucleus of the host cell from the intestine of a calf. Residual bodies, lipid-like structures, mitochondria, endoplasmic reticulum, and plastic granules ("labyrinth-like bodies" of Scholtyseck) that eventually form the oocyst wall were observed and electron micrographs were made with enlargements of up to 150,000 times.

Three calves were used to build up pure cultures of Eimeria auburnensis and these were used in 3 calves to obtain tissues of life history stages as shown by electron micrography. Two immature schizonts were located and photographed.

(Auburn, Alabama) (ADP-bl-23(Rev.))

B. Clinical and Physiological Aspects of Roundworm Parasitism in Cattle including Anthelminitc Treatment.

The School of Veterinary Medicine, University of California, Davis, under a cooperative agreement with the USDA, reports on their investigations as follows:

Anthelmintic Studies - (a) Maretin as an oral drench at 50 mg/kg and 75 mg/kg was quite effective in clinical parasitism of cattle. Co-Ral at 2 mg/kg/day for 6 days in feed was also very effective. (b) Tetramisole in lambs was very effective as an anthelmintic at 10 and 15 mg/kg. Activity was marginal at 5 mg/kg. (c) Thibenzole is very effective when used in a prophylactic program of parasite control in cattle. Larvicidal activity, however, cannot be depended on for control of type II ostertagiosis.

Toxicological Studies - Organophosphate toxicity in cattle has been studied under varied conditions in California. Conclusions are that toxicity is unpredictable, better grades of cattle are more susceptible, and the problem is primarily in northern counties.

A genetic resistance to paralytic toxicity of Haloxon in sheep has been confirmed and shown to be associated with the presence of an A-esterase in plasma. The presence of the esterase is inherited as a single dominant gene.

Development of an Experimental Model for Laboratory Study of Physiological Alterations - The course and pathology of Obeliscoides cuniculi infection in rabbits has been very similar to that of ostertagiosis in cattle and sheep.
(Davis, California) (ADP-b1-25(Rev.))

C. Investigations of Trichomonad Parasites.

The Division's Regional Animal Disease Laboratory at Logan, Utah, reported that, in antigenic comparison of 6 strains of Trichomonas foetus, antisera against these strains produced in rabbits had previously had relatively high homologous and heterologous agglutinating titers. In similar precipitating reactions, antigen-antibody reactions occurred against ingredients from the medium in which the trichomonads were grown. This finding indicated that the rabbits were at least partially immunized against these ingredients. Reruns of agglutinating titers, considering these factors, revealed that the trichomonads were not surface-contaminated to any great degree by these ingredients and that the original titer determinations were valid. Precipitating reactions with the rabbit antisera and trichomonad antigens, however, revealed that antibodies to bovine serum proteins were produced that were ingested by the trichomonads from their culture medium. This determination was made by a refinement of the double diffusion method previously used. By using this micro-method, approximately 3 times as many antigen-antibody systems were detected as previously found by macro-methods. Final analysis, separation and comparison of these antigens is now underway. If it can be recognized that strains of T. foetus are highly dissimilar antigenically, they might be also dissimilar with respect to pathogenicity, a point of main concern to many at present.

There still exists considerable difference of opinion as to the best method of diagnosis of bovine trichomoniasis. For this reason a compilation of results of tests made at this station by the douche method on known infected bulls was made. This large number of tests made for years under various field conditions clearly reveals that the douche method is superior to other methods.

(Logan, Utah) (ADP-b1-26)

D. Host-Parasite Relationships of Intestinal Worms, Cooperia species, in Cattle.

Reported research from the Division's Regional Animal Disease Laboratory, Auburn, Alabama, showed that acquired immunity of calves to Cooperia pectinata and C. oncophora provides some immunity against subsequent infection with C. punctata, with C. oncophora providing the greater protection.

Three calves were infected with C. oncophora, C. pectinata, and C. punctata, respectively. When the parasites were in the parasitic fourth-stage the calves were necropsied and the worms were picked from the intestinal contents and segregated according to species and sex. Laparotomies were performed on 4 helminth-free calves and the worms were injected into the duodenum of each animal via hypodermic syringe. One calf received male C. oncophora and female C. punctata. A second calf received the reciprocal of this cross. A third calf was given male C. pectinata and female C. punctata and a fourth calf the reciprocal of this cross. Progeny of these crosses in the form of parasitic third-stage larvae were used, in turn, to infect helminth-free calves. No hybrid parasites were found at necropsy.

A prosthetic device was designed, constructed, and surgically placed in the duodenum of a bovine animal to facilitate sampling of duodenal fluids. This device was made of plexiglass and had a "nut and bolt" construction which allowed tightening upon reduction of post-operative swelling. The intestine was drawn to the body wall by the prosthesis and as healing progressed adhesions affixed the small intestine and peritoneum to the body wall. A short plastic tube was attached to the device to aid sampling. Several weeks after surgery, the prosthesis became dislodged because of increased muscular activity of the animal. However, sampling procedures could be maintained by use of a plastic tube through the fistula.

Three groups of 8 guinea pigs were infected with parasitic third-stage larvae of either C. punctata, C. oncophora, Nematospiroides dubia or Oesophagostomum radiatum. Infective larvae were administered either as sheathed or exsheathed larvae. Three days before administration of infective larvae, guinea pigs in each group were given by injection either a saline suspension of cortisone acetate or saline minus cortisone at the rate of 45.2 mg/kg body weight. Some were left as controls. Injections of cortisone or saline were given each day until necropsy. Guinea pigs were killed at varying intervals after infection. No parasites were found in guinea pigs infected with C. punctata, C. oncophora, or O. radiatum. Parasitic worms were found in all animals infected with N. dubia but animals receiving cortisone had no more worms than those given saline or those used as controls. There was no difference in numbers of worms observed in animals infected with either sheathed or exsheathed N. dubia.

(Auburn, Alabama) (ADP b1-27)

E. Effects of Level, Rate, and Period of Exposure to Larvae on the Establishment and Pathogenesis of Gastrointestinal Nematode Parasites of Cattle.

The Beltsville Parasitological Laboratory reports as follows: In tests resulting in mild to moderate infections, level and duration of exposure to larvae affected the establishment and turnover of the gastrointestinal nematode burdens of calves. The largest numbers of worms were established in the calves exposed at the highest levels and these calves also lost the greatest number of parasites. However, the worm egg output was similar at the various levels of exposure. The loss of worms was due to their senescence and to the development of resistance to them by the host. Although individual calves differed in susceptibility to infection and ability to develop resistance to the different species, resistance generally increased with the duration of exposure to infective larvae. Some retardation of development was noted in 1 species following exposure of calves for only 3 days. Resistance to some species of nematodes was greater than resistance to others. These findings indicate that selective breeding of cattle for resistance to parasites may be a fruitful line of research.

(Beltsville, Maryland) (ADP b1-28 and b1-37)

F. Etiology and the Immune Response of Cattle to Winter Coccidiosis.

At the Division's Regional Laboratory at Logan, Utah, work was completed on the results of continuous low-level inoculations of Eimeria bovis in calves and the effects of bovine coccidiosis on the blood serum sodium and potassium levels in calves. It was ascertained that calves fed 10, 100, 500, 1,000, or 15,000 oocysts/day for up to 62 days, became immunized to infections resulting from the daily inoculations. Calves given as few as 110 oocysts over an 11-day period developed immunity sufficient to protect them from severe clinical signs of coccidiosis resulting from inoculations with 500,000 oocysts.

In calves 4 to 8 weeks old, Eimeria bovis caused only minor changes in the blood serum sodium and potassium levels unless signs became severe or the calf became moribund. In these instances, the potassium levels rose and the sodium levels decreased 6 to 8 hours before death. At death, the potassium levels were about 8 mEq/l. while the sodium levels were about 105 mEq/l. Normal levels are about 5.0 and 140 mEq/l. Cellular death caused by toxins and dehydration, associated with the moribund condition, probably caused the alterations in the serum electrolytes.

(Logan, Utah) (ADP b1-29)

G. Bovine Anaplasmosis.

The Beltsville Parasitological Laboratory reports that improved methods of fixation show that the Anaplasma body has more internal structure than previously recognized, when viewed in the electron microscope. There are clearly defined 3-ply membranes that closely resemble the covering (pellicle) of protozoa.

The soluble antigen of A. marginale is believed to be red blood cell protein that has been metabolized and altered by the parasite. Little, if any, protection was produced when this material was used as an immunizing agent. Large quantities of the soluble antigen inoculated intravenously were not toxic.

Therapy trials with an experimental drug have shown a very marked inhibitory effect of the drug on the Anaplasma organism.

A summary of the work on experimental transmission of bovine anaplasmosis by ticks is summarized in the report by Entomology Research Division.
(Beltsville, Maryland) (ADP b1-30)

Investigations on anaplasmosis, piroplasmosis, and babesiellosis of cattle were conducted under a PL 480 grant to the School of Veterinary Parasitic Disease, Montevideo, Uruguay.

Electron micrographs have been made of the anatomy of uninfected ticks to provide the necessary background for the recognition of pathogenic alterations by Anaplasma. Babesia has been identified in the lumen of the gut of ticks.

In studies on tick development in vitro, it was reported that growth from larva to nymph, does not offer difficulties and methods have been greatly improved. It has become possible to obtain any amount of nymphs desirable to perform different experiments. Feeding through a dialysis sac seems to be possible, although observations are not yet definite.

Controlled field studies are underway on cattle immunized with A. centrale and B. bigemina.
(Montevideo, Uruguay) (PL 480 S9-ADP-1)

H. Histochemistry of Gastrointestinal Nematodes of Cattle.

Research work at the Division's Regional Animal Disease Laboratory at Auburn, Alabama, was reported as follows: Studies have been confined to confirming the results reported in earlier annual reports and to developing

techniques for studying penetration into the wall of the small intestine of the mouse by larvae of the nematode Heligmosonum skrijabini (=Nematospiroides dubia).

Within the limitations and range of the techniques employed (see preceeding annual reports), the histochemistry of host response to the larvae of the nodular worm, Oesophagostomum radiatum, within its tissues has no qualities peculiar to the vermian nature of the penetrator. Methods other than strictly histochemical ones will have to be used in studying the enzymology of larval penetration by Heligmosomum skrjabini and, by extension, O. radiatum.

(Auburn, Alabama) (ADP b1-32)

I. Worm Parasites of Cattle on Irrigated Pastures and on High-Rainfall Areas of the Southwest.

The Division's Regional Animal Disease Laboratory at University Park, New Mexico, reported as follows:

Stephanofilariasis in New Mexico Cattle: Examinations conducted in slaughterhouses in eastern New Mexico showed that 95% of cattle over 5 years of age had skin lesions on the ventral surface caused by stephanofilarial worms. In research conducted at the University Park Field Station, these worms were transmitted by horn flies. The average size of the areas of the skin involved was 22 square inches. The lesions make sizable portions of the hide unsuitable for leather; they are also sites where secondary bacterial infection may occur. Teats are involved in some cases. This involvement raises questions about a possible relationship with mastitis and rejection of calves by their mothers. These problems will be investigated in future work.

Parasites of Elk in New Mexico: Elk occur in close association with cattle in many parts of the West. Therefore, information concerning the parasites common to these animals may assist in preventing parasitism. Exploratory examinations made so far have shown that elk harbor one species of tick that also parasitizes cattle, one species of liver tapeworm in common with sheep, and one species of lungworm and one species of gastrointestinal nematode. Specific identities of all are unknown as yet.

(University Park, New Mexico) (ADP b1-33)

J. Effect of Stocking Rate and Rotational Grazing on Internal Parasitism in Beef Cattle.

This work was done at Experiment, Georgia, under the auspices of the Division's Regional Animal Disease Laboratory at Auburn, Alabama. The third year's test was completed to determine the effect of stocking rate and

rotational grazing of steers on their parasitic populations. Two lots of winter temporary forage were maintained at the same stocking rate throughout the grazing season - one was grazed continuously and the other was grazed on a four-way rotational system. A third lot was also grazed rotationally, but the stocking rate varied with the condition and carrying capacity of the pasture at any time. The average stocking rates for lots 1, 2, and 3 were 1.1, 1.1, and 1.4 animals per acre, respectively. Average daily gain was 1.91, 1.86, and 1.60 lbs. for the 3 lots. The steers from the rotationally grazed pasture with the variable stocking rate harbored the largest number of worms. Contrary to results obtained during the last 2 years, the animals from the continuously grazed pasture had more worms than those rotationally grazed.

The first year study on the effects of 3 stocking rates and 3 levels of parasitic infection on beef cattle was concluded. In general, results indicate that an increase in the stocking rate of the pastures and on the level of parasitic infection affected the performance of the cattle. The average daily gain, slaughter grades, and carcass grades were reduced as the stocking rate was increased. Reduction was also observed as the degree of parasitism was increased. The parasites obtained from the slaughter animals are being counted and identified at present.

(Experiment, Georgia) (ADP b1-34)

K. Effect of Host Diet on the Bionomics of the Preparasitic Stages of Nematodes in Cattle Feces.

This work was done at Experiment, Georgia, under auspices of the Division's Regional Animal Disease Laboratory at Auburn, Alabama. The antioxidant used in commercial feeds (Santoquin, Ethoxyquin, or E.M.Q.) had an inhibitory effect on nematode larval development in calf feces. Increasingly fewer nematode larvae were recovered from fecal cultures as the quantity of E.M.Q. fed to infected calves was increased from the amount commonly used as a preservative (1/4 lb./Ton) up to 100 times (100x) that amount. In general, this reduction was true for Cooperia pectinata, C. punctata, Trichostrongylus colubriformis, T. axei, Ostertagia ostertagi, and Oesophagostomum radiatum. There was a statistically significant reduction in the numbers of infective larvae recovered from cultures of feces from calves with T. axei or C. pectinata infections when varying amounts of an antioxidant, E.M.Q. were added to the cultures. Similar reductions were observed when E.M.Q. was added to cultures of feces from a calf with a mixed natural infection.

(Experiment, Georgia) (ADP b1-35)

L. Life History and Host-Parasite Relationships of Nematode Parasites.

Research on nematode parasites can be conducted in laboratory animals more economically than in ruminants. To determine detailed life history, morphogenesis, and host-parasite relationships, work was pursued on Trichostrongylus affinis in rabbits at State College, Mississippi under the auspices of the Division's Regional Animal Disease Laboratory, Auburn, Alabama.

One experiment indicated that 40,000 infective larvae of T. affinis can produce death in rabbits 6 to 8 weeks old. Based upon weight, the peak of the ill effects of the infection was reached on the eleventh day; however with infections of 20,000 larvae and above, a decrease in weight gains coincided with the emergence of the worms from the tissues on the fourth day.

Two studies involving rabbits infected with Trichostronyglus affinis have demonstrated that rabbits will acquire a resistance or partial immunity through a previous infection. Rabbits given 3000 infective larvae and then given a challenge dose of 6000 larvae at a later date harbored significantly fewer worms than rabbits that received the challenge dose of 6000 infective larvae only.

(State College, Mississippi) (ADP b1-36)

M. Environmental Factors Influencing Parasites and Parasitic Diseases.

Under a PL 480 Grant to the School of Veterinary Medicine, University of San Marcos, Lima, Peru, research is being conducted on environmental factors influencing parasites and parasitic diseases of economical importance in ruminants (cattle, sheep, alpacas). This study is nearing completion and resulting data is presently being compiled in a form which will be useful and of practical value.

(Lima, Peru) (S8-ADP-1)

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Frandsen, John C. 1966. Changes in the connective tissue of the bovine ileal wall associated with infection by the larval nodular worm, Oesophagostomum radiatum (Rudolphi). Trans. Am. Micr. Soc. 85:160-161.

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AREA NO. 12 - PARASITES AND PARASITIC DISEASES OF SWINE

Problem. Parasitic diseases have been estimated to cost the swine industry of the United States at least $200 million annually. These diseases for the most part are cosmopolitan. Subclinical infections are the most frequent type and the most costly, yet they are generally so difficult to recognize that they often are overlooked entirely. Diagnosis is difficult, and successful treatments for many of these parasitisms are not available. Moreover, management practices to avoid the spread of parasitisms and to control them are often ineffectual. The problem is to develop, through a planned, balanced program of basic and applied research, knowledge for preventing, controlling, or eradicating parasitic diseases so as to provide for healthy swine, insure adequate supplies of parasite-free pork for an expanding population, avoid or minimize economic losses caused by these diseases, and thereby contribute to a prosperous agriculture, a sound national economy, a high standard of living, and a healthy population.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving parasitologists, veterinarians, biochemists, microbiologists, and pathologists engaged in basic and applied research in this problem area. Research is being conducted on the following diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 5.2 scientific man-years. This effort is divided among sub-headings as follows:

Pathogenic Role of the Intestinal Roundworm 0.1 under a cooperative agreement with the Nebraska Agricultural Experiment Station, Lincoln.

Investigations of Trichinella spiralis 1.0 at the Beltsville Parasitological Laboratory and through a PL 480 grant to the Polish Academy of Science, Warsaw, Poland.

Strongyloides ransomi Infections in Baby Pigs 1.0 at the Swine Parasite Laboratory, Tifton, Georgia.

Biochemical and Other Aspects of the Host-Parasite Relationship in the Development and Severity of Helminthiasis in Swine 2.0 at the Beltsville Parasitological Laboratory.

Life Cycle of the Nodular Worm of Swine 0.5 at the Swine Parasite Laboratory, Tifton, Georgia.

Infection of the Dung Beetle, Phaneaus vindex, with Larvae of the Thick Stomach Worms 0.5 at the Swine Parasite Laboratory, Tifton, Georgia.

Swine Kidney Worms 0.1 under a cooperative agreement with the North Carolina Agricultural Experiment Station, Raleigh.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 2.9 scientific man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Intestinal Roundworm (Ascaris suum).

In cooperative research at the University of Nebraska, sows were hyperimmunized with repeated oral doses of infective eggs of Ascaris suum. Baby pigs from the immunized sows made better average daily weight gains than baby pigs from nonimmunized sows.

Previous studies demonstrated that addition of aminopeptidase, an enzyme isolated from adult A. suum, to the drinking water resulted in mice becoming more susceptible to parasitism. The results of the study this year were in agreement. After orally dosing mice with infective eggs of A. suum, a significantly larger number of larvae were recovered from the lungs and liver of the mice that received the enzyme in the drinking water than from the control mice.

In an effort to determine if this increase in susceptibility was elicited by the presence of specific parasite proteins or by foreign proteins from other sources, bovine gamma globulin was placed in the drinking water. The results of the study suggest that the increase in susceptibility was elicited by specific parasite proteins. Fewer larvae were recovered from the mice which received bovine gamma globulin in the drinking water than from controls.

Eight to 12-week-old hysterectomy-derived, colostrum-deprived, antibody-devoid pigs evidenced a lack of natural resistance to infection by migrating nonirradiated larvae of A. suum. Severe clinical signs of ascariasis were manifested by 15 pigs after oral dosing with 50,000 nonirradiated infective eggs of A. suum. Three pigs died 9 to 10 days postinfection. However, in 8 of 11 pigs dosed with 50,000 X-irradiated (eggs of A. suum were treated with 40,000 or 70,000 roentgens of X ray) infective eggs of A. suum, only a slight increase in rate of respiration occurred because a smaller number of larvae reached the lungs.

Eight hysterectomy-derived, colostrum-deprived, antibody-devoid pigs, which were orally dosed with 50,000 nonirradiated infective eggs of A. suum and then given a challenge dose of 50,000 or 200,000 nonirradiated infective eggs, developed little or no resistance to reinfection by migrating larvae of A. suum. After receiving the challenge dose of nonirradiated infective eggs, the 8 pigs developed severe clinical signs of ascariasis and 2 pigs died. Eight pigs orally dosed with 50,000 X-irradiated infective eggs did develop resistance to reinfection. Only a slight increase in respiration rate was observed in 3 of the 8 pigs following the challenge dose of 50,000 or 200,000 nonirradiated infective eggs.

When infective eggs of A. suum are treated with X ray before dosing pigs, a reduction occurs in the number and length of larvae migrating to the lungs and liver. When pigs, which have been orally dosed with X-irradiated or nonirradiated infective eggs, are given a second dose of nonirradiated infective eggs, the larvae migrating to the lungs from the second dose will be reduced in length.

(Lincoln, Nebraska) (ADP b2-12(Rev.))

B. Trichinosis (Trichinella spiralis).

Investigation of the fluorescent antibody technique as an aid to the serologic diagnosis of trichinosis in swine was initiated during the year. Antigen was made by subjecting trichinous meat to a temperature of 0° F for about 10 days and digesting the tissue and cysts. The transparent cuticle surrounding the dead worm material is to be used as the antigen in the indirect fluorescent antibody test. The cysts surrounding the trichinae in muscle tissue will also be investigated as a possible binding site.

Serum was obtained weekly from 7 pigs for 12 weeks. The first blood samples were drawn on the day 4 pigs were infected with trichinae. Two pigs were each fed 144 trichinae/lb. of body weight and 2 received 576 larvae/lb. of body weight. Three pigs were kept as uninfected controls. The blood was stored overnight in a refrigerator and the serum was separated from the clot by centrifugation the following morning. About 15 ml. of each sample was placed in serum bottles, stoppered, and stored in a freezer at a temperature between 0° and -10° F. Serum samples were collected for 12 weeks.

Antispecies globulin was prepared by the repeated intravenous injection of rabbits and chickens with globulin obtained from swine serum. The chickens and rabbits were killed 12 weeks after the beginning of the series of injections, the serums collected and stored in the freezer. Precipitation with rivanol and conjugation of the globulin with fluorescein isothiocyanate should result in several lots of labeled, antispecies globulin for use in future FA tests with hogs.

Trichinella antigens were produced from fresh larvae, frozen larvae, and from cysts. One trial has been conducted with cysts fixed in a solution of 10% formalin and 0.5% bovine serum for 5 minutes. The test serum was then added to the washed cysts and they were then incubated 30 minutes at room temperature on a slide rotator. After a second washing, the cysts were treated with 0.1 ml. of the commercial antispecies globulin for 15 minutes on the rotator. The cysts fluoresced under ultraviolet light.

(Beltsville, Maryland) (ADP b2-15 (Rev.))

Investigations on trichinellosis are also being conducted under a PL 480 grant to the Polish Academy of Science, Warsaw, on the epidemiological, epizootiological, and immunological aspects of this disease to establish information on the incidence of Trichinella spiralis in people and domestic and wild animals throughout the country. Allergic tests for diagnosis of the disease are being assessed. Other studies indicate that the intestinal flora in the host's digestive tract may affect the invasive ability of the larvae.

(Warsaw, Poland) (E21-ADP-9)

C. Strongyloides ransomi Infection in Baby Pigs.

Previous observations on the effects of Strongyloides ransomi infection of weaned pigs have been made largely on pigs harboring the normal nematode fauna of farm-raised pigs of the area. The management of the pigs is considered to be very good. From the time the pigs are a few days old, they have access to a balanced creep feed and once weaned they are full-fed a fortified high-protein ration.

Interactions can be expected among the variety of parasites in the same environment of the pig's gut. What effects a fortified high-protein diet may have in helping pigs overcome deleterious effects of parasitic nematodes is not well known. An attempt was made to remove all parasites from a group of pigs fed on corn and tankage.

Twenty Hampshire pigs raised on the farm were selected and assigned to pens on the basis of weight, sex, and litter origin. Each pig was given 2 grams of thiabendazole suspended in 2 ounces of liquid and then each pen of 5 pigs was offered 64 additional grams of the active drug mixed in a small amount of feed. Feed had been withheld from all the pigs for the previous 4 1/2 hours. Theoretically, each pig received 400 mg./kg. of body weight, but not less than 100 mg./kg. of body weight.

All fecal samples taken from each of the pigs were negative for worm eggs for the ensuing 32 days, at which time 10 pigs were each exposed to 2 million infective larvae of S. ransomi. Exposure was by the usual method of placing larvae suspended in a small amount of water on the cleansed skin of the iguinal region. Each pig was held immobile for 10 minutes to give the larvae a chance to penetrate the skin.

Two weeks later the same pigs were exposed to an additional 1 million larvae of S. ransomi. The outside of the right ear was used as the site of exposure. Larvae suspended in a small amount of water were placed on a 5 mm. circular piece of cheesecloth made up of 12 layers. The cloth pad was firmly taped to the ear with masking tape and left for at least 15 minutes.

Most of the pigs did not show a second sharp peak in Strongyloides egg production. The maximum worm egg counts were relatively small for the number of larvae to which the pigs were exposed.

All pigs were fed the same inadequate ration of ground yellow corn and tankage containing 16% crude protein for the entire experiment.

The average daily gain of both groups of pigs was poor; the infected group gained 0.3 lb. less per day than did the controls. The most significant difference was in the feed conversion. The infected pigs required 0.63 lb. more feed per pound of gain than did the controls.

(Tifton, Georgia) (ADP b2-17)

D. Biochemical Aspects of Host-Parasite Relationships.

Gas-liquid chromatographic studies show that male and female Ascaridia galli, the large intestinal roundworm of chickens, differ in their neutral lipid composition. Of 4 as yet unidentified methyl ester fractions, constituting 0.43 to 1.5% of the total, 3 were found exclusively in females and 1 exclusively in males. Three times as much myristic acid and an unidentified ester with a carbon number C-17.2 were found in males as were found in females. On the other hand, female worms contained from 1 1/2 to 2 1/2 times as much palmitic, oleic, and linoleic acids as males. These studies are the prelude to others dealing with the physiological and pathological changes that occur in the host-parasite relationship.

(Beltsville, Maryland) (ADP b2-18)

E. Nodular Worm of Swine, Oesophagostomum brevicaudum.

Eggs of Oesophagostomum brevicaudum developed faster and the larvae survived longer in feces-moss cultures than in feces-charcoal cultures. Cultures were incubated at 15, 20, 25, 30, and 35° C.

The maximum percentage of development was at 35° C (41%), but survival was poor after the 7th day. At 14 days, only 78% of the larvae survived.

At 25° C , 36% of the eggs developed. At 14 days, 94% of these larvae still survived.

In general, development was faster and survival poorer in moss cultures than in charcoal cultures.

The various stages of the parasitic and free-living cycles of O. brevicaudum have been collected. Morphological characters observed can be used to differentiate the infective larvae of this species from those of the other swine nodular worms, O. dentatum and O. quadrispinulatum.

(Tifton, Georgia) (ADP b2-19)

F. Infection of the Dung Beetle, Phanaeus vindex, with the Larvae of the Thick Stomach Worms of Swine.

During the summer of 1965, a former swine pasture of about 0.6 acre was marked off to provide 45 "trap sites." Each of 3 "trap sites" was baited daily for 5 days with 100 cc. of fresh swine feces. The feces were obtained from a pig infected with the thick stomach worm, Physocephalus sexalatus. Some of the "trap sites" were baited again after a lapse of 15 weeks.

The habits of the dung beetle, Phanaeus vindex, are such that being attracted to a fresh swine stool, the beetles land a short distance from their target and cover the remaining distance on the ground. At the edge of the stool, the beetles dig underneath the pile.

The beetles drag chunks of feces down into the tunnels only a short distance for food and much deeper for brooding chambers.

Most of the tunnels inspected go straight down at a slight angle to the perpendicular. Masses of dung are packed at the end of short tunnels for use as food. Other masses are rolled with a 1/4-inch external layer of clay until smooth balls are formed with a slight elevation at one pole. A single egg is laid by the female in a small depression of the elevation. The larva ensuing from the egg burrows into the dung ball. As the larva feeds and grows, the brooding chamber becomes lined with the larval excrements and shed larval skins.

(Tifton, Georgia) (ADP b2-20)

G. Swine Kidneyworm (Stephanurus dentatus).

Parasite-free pigs obtained by a modified specific-pathogen-free program were infected with single oral doses of 50,000 infective larvae of S. dentatus. Uninfected pigs were maintained as controls. Blood samples were taken every 4th day and determinations made of changes in histamine by the technique of Noah and Brand. No consistent correlation was observed in fluctuations in histamine values and liver changes produced by the larvae.

Liver function values were determined on these pigs by use of the bilirubin test as described by Malloy and Evelyn. Inconsistent correlation was found between liver function values and liver destruction as determined at necropsy.

A new series of organo-phosphate compounds became available to the leader this year. Naturally-infected sows were purchased at a local sales barn. The test compound was given to the sows in single oral doses. Urine samples were collected pre- and post-treatment to determine influence on ova production. None of the test compounds deterred ova production within a nontoxic level.

(Raleigh, North Carolina) (ADP b2-21)

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AREA NO. 13 - PARASITES AND PARASITIC DISEASES OF SHEEP AND GOATS

Problem. The cost of parasitic diseases to the sheep and goat industry of the United States is estimated to be in excess of $45 million annually. Disorders caused by parasites are ubiquitous, generally insidious and often overlooked entirely. Diagnosis is difficult, and successful treatments for many of these diseases are not available. Moreover, management practices to avoid spread of parasitisms and to control them are often ineffectual. The problem is to develop, through a planned, balanced program of basic and applied research, knowledge for preventing, controlling or eradicating parasitic diseases so as to provide for healthy animals, insure adequate supplies of high quality lamb for an expanding population, avoid or minimize economic losses caused by these diseases, and thereby contribute to a prosperous agriculture, a sound national economy, a high standard of living, and a healthy population.

USDA AND COOPERATIVE PROGRAM

The Department has a continuous long-term program involving biochemists, parasitologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of parasites and parasitic diseases of sheep and goats. Research is being conducted on these diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 8.7 scientific man-years. This effort is divided among sub-headings as follows:

Gastrointestinal Nematodes 1.1 at the Beltsville Parasitological Laboratory, and under a cooperative agreement with the Kentucky Agricultural Experiment Station, Lexington.

Life Histories, Biology, Pathogenesis, and Control of Helminth Parasites of Sheep in the Southwest 1.0 at the University Park, New Mexico, field station, and through informal cooperation with the New Mexico Agricultural Experiment Station, University Park.

Life Cycles of Sheep Coccidial Parasites 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Effect of Intestinal Roundworms on the Tensile Strength and Sulfur Content of Wool 0.1 under a cooperative agreement with the North Dakota Agricultural Experiment Station, Fargo.

Immunity to the Intestinal Worm, Trichostrongylus colubriformis 1.5 at the Regional Animal Disease Research Laboratory, Auburn, Alabama.

Control of the Common Sheep Scab Mite, Psoroptes ovis 0.5 at the Parasite Research Laboratory, Albuquerque, New Mexico.

Chemical Control of Sheep Nose Bot, Oestrus ovis 0.5 at the Parasite Research Laboratory, Albuquerque, New Mexico.

Biology and Control of Psorergates ovis, the Australian Itch Mite of Sheep 0.5 at the Parasite Research Laboratory, Albuquerque, New Mexico.

Pathobiology of Laboratory and Field Strains of Psoroptes ovis 0.5 at the Parasite Research Laboratory, Albuquerque, New Mexico.

Overwinter Survival of Parasitic Nematode Larvae on Mississippi Pastures 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama, and in cooperation with the Mississippi Agricultural Experiment Station, State College.

The Biology and Control of Liver Flukes 1.0 at the Parasite Research Laboratory, University Park, New Mexico, and a PL 480 grant with the Veterinary Faculty, Ankara University, Ankara, Turkey.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 3.9 scientific man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Gastrointestinal Nematodes and Nematodiasis of Sheep.

The results of two experiments in which 24 lambs were used indicated that colostrum-deprived, parasite-free lambs were more susceptible to the effects of gastrointestinal parasitism than lambs raised to weaning age with their dams. In one experiment, all of the lambs were exposed to natural infection with miscellaneous internal parasites while grazing a pasture contaminated with infective larvae. In the other experiment, the lambs were artificially infected with comparable numbers of larvae of the large stomach worm, Haemonchus contortus. In both instances, the colostrum-deprived, parasite-free lambs had lower hematocrit levels and made poorer weight gains than the lambs raised with their dams, although their worm loads were similar to or less than those of the last-mentioned group.

(Beltsville, Md.) (ADP b3-16 Rev.)

Previously, it was reported that certain strains of Haemonchus contortus in Kentucky are tolerant to phenothiazine (N.F.) and more recently to thiabendazole. Several strains have been isolated and maintained in donor lambs in the laboratory. Controlled tests were completed in October, January, and May in order (1) to evaluate more fully the efficacies of

thiabendazole and phenothiazine (2-3 μ, N.F. and purified) against strains A, B, C, and X (2) to determine if strains A, B, and C have retained susceptibility or tolerance to thiabendazole or phenothiazine (N.F.) over the several years that they have been maintained in donor lambs in the laboratory (Strain X is a recent isolate). Strain A is considered phenothiazine (N.F.) susceptible and strain B phenothiazine (N.F.) tolerant. Strains C and X are thiabendazole tolerant. Single controlled tests were carried out with strains A, B, and C, and two with strain X. Larvae were cultured from feces of donor lambs with pure infections of the appropriate strains and administered with a dose syringe equipped with a Whitlock nozzle. The number of larvae given each lamb in each test was: Strain A, 2,640; strain B, 5,100; strain C, 5,700; strain X, experiment No. 1, 5,100; and experiment No. 2, 3,000. In each test, about 1 month after infection, treatments were made as follows: thiabendazole at 50 mg/kg, phenothiazine, 2-3 μ, N.F., at 220 mg/kg, and phenothiazine, 2-3 μ, purified, at 220 mg/kg. There were 2 lambs allotted to each of the treated groups except in experiment No. 2, with strain X where there was 1 lamb. From 1 to 3 lambs were placed in the untreated control group for each test. The lambs were allotted to groups according to body weight and pretreatment fecal egg counts. Individual doses of each compound were weighed, mixed with water, and administered with a dose syringe equipped with a Whitlock nozzle. All lambs were killed about 1 week after treatment. The abomasal contents and rinses of water were examined under an X3 magnifying glass after washing in a 30-mesh sieve. Posttreatment egg counts were made from feces collected at necropsy. Strain A was susceptible to all treatments. Strain B showed resistance to phenothiazine (N.F.) but was susceptible to the other two treatments. Strain C showed resistance to all treatments. Strain X showed resistance to thiabendazole and phenothiazine (N.F.) but was susceptible to purified phenothiazine.

(Lexington, Kentucky) (ADP b3-16 Rev.)

B. Life Histories, Biology, Pathogenesis, and Control of Several Helminth Parasites of Sheep in the Southwest.

Effect of Age on the Immune Response of Lambs to Experimental Haemonchosis. Large stomach worms (Haemonchus) occur in cattle, sheep, and other ruminants in many parts of the world and are extremely harmful parasites. In New Mexico, Haemonchus strains from wild sheep and from antelope are considerably less damaging to domestic sheep than strains originating in sheep. At present, we are using a strain from antelope in an effort to immunize domestic lambs against sheep strain haemonchosis. Research just completed indicates that 2½-month-old lambs inoculated with antelope strain worms and then given a drug to remove the immunizing infection are slightly immune but that 5-month-old lambs similarly processed are markedly immune. The role the antelope strain might play in immunizing sheep under practical conditions remains to be determined.

Electrophoretic Studies on Haemonchus Infections. Electrophoretic studies on the plasma of lambs infected with both the antelope and sheep strains of Haemonchus showed an increase in gamma globulin, a fraction with which an immune response is often associated. However, this increase was not directly related to the other evidence of immunity, which suggests that immunity in haemonchosis may be due to a local tissue reaction, some elements of which enter the circulating blood. This information helps to give us a better understanding of the immune state in this very important parasitic disease.

The Taxonomy and Life History of Nematodirus oiratianus. It has been determined that certain intestinal nematodes occurring in a wide variety of ruminants in the West and Southwest and previously tentatively identified as Nematodirus lanceolatus, a species known only in Argentina, are in reality N. oiratianus, a species heretofore known only in Asiatic Russia. Preliminary observations on the life history have revealed new information which should be of value in preventing and controlling infections with this parasite.

Parasites of Jack Rabbits in New Mexico. Examinations of rabbits inhabiting sheep range in New Mexico have turned up a wide variety of parasites, but only one - Trichostrongylus colubriformis - is known to parasitize domestic stock. Probably rabbits play a part in the propagation of this parasite among sheep and cattle.

(University Park, New Mexico) (ADP b3-18)

C. Life Cycles of Eimeria ahsata and E. crandallis (Sheep Coccidia)

Additional studies were made on sections of tissues infected with Eimeria ahsata because intranuclear stages as well as second generation schizonts had been found.

The second generation schizonts were mostly in the epithelial cells lining the crypts of Lieberkühn, except for 2 that were found in the lumen of a gland and in the interstitial spaces between the crypts.

(Auburn, Alabama) (ADP b3-19)

D. Effect of Intestinal Roundworms on Wool Quality.

In studies conducted to determine the effect of gastrointestinal roundworms on sheep, there was an apparent reduction in the blood glucose and B-hydroxybutyrate but apparently no effect on the pyruvate, acetone, or pH of the blood. The lambs were receiving a high level of nutrition which might obscure some effects of parasitism.

Future studies will determine whether changes in these blood components are more marked when the nutritional level is lower.

(Fargo, North Dakota) (ADP b3-20)

E. Immunity to the Intestinal Worm, Trichostrongylus colubriformis.

Infections of guinea pigs with Trichostrongylus colubriformis were terminated chemically 2, 5, and 12 days after oral inoculation with normal infective larvae. Parasitic stages of development on these days were: third, fourth, and fifth, respectively. Infections consisting of only parasitic third-stage worms resulted in a high degree of immunity to reinfection, fourth-stage worms provided no significant additional protection, and infections terminated after worms had reached the fifth stage resulted in almost complete immunity.

Chemically terminated parasitic third- and fourth-stage infections failed to provide immunity against reinfection in lambs.

The immune response in guinea pigs was directed against parasitic third- and fourth-stage worms of the challenge inoculations, and immunity was not fully expressed, as reflected by maximum elimination of worms, until 9 days after challenge.

Infective third-stage larvae of Trichostrongylus colubriformis can be stored on moist filter paper at 4° C. for as long as 12 months without decrease in viability and infectivity; storage for 15 and 18 months results in marked decreases.

(Auburn, Alabama) (ADP b3-21)

F. Control of the Common Sheep Scab Mite, Psoroptes ovis.

Four potential miticides were screened against P. ovis, as follows: Prolate, Malathion, Quinothion, and SD 8447.

One of these, SD 8447, has a history of effectiveness against Boophilus spp. on Mexican cattle, but failed in an initial test against P. ovis. Another, Quinothion, was successful on initial test, but presents stability problems in the field, and was not further investigated. The 2 remaining products, Prolate and Malathion, were exposed to cage-vat and extensive trough-vat tests, and proved eminently successful. Both are broad-spectrum ectoparasiticides, are relatively nontoxic to hosts, and merit continued investigation. Prolate, in particular, has been highly effective against Boophilus spp. on Mexican cattle.

The screening trials all involved sheep heavily infested with highly pathogenic, summer-resistant strains of P. ovis.

(Albuquerque, New Mexico) (ADP b3-22)

G. Chemical Control of Oestrus ovis.

The apparent fumigatory effect of Vapona (DDVP)-impregnated plastic strips against Haematopinus eurysternus, the big gray louse of cattle, was so rapid and so great that it was decided to test its ability to protect sheep against the strike of adult female Oestrus ovis flies.

Thirty aged ewes, all originally from the same range flock, were selected as test animals. On August 9, 1965, each of the 30 sheep was weighed and treated intramuscularly with 52% dimethoate at the rate of 25 mg/kg. The sheep were then randomly divided into 3 groups of 10 each. One group, designated dimethoate controls, was not further treated. One group, designated Vapona controls, was fitted with simulated Vapona collars made of 1-inch cotton webbing wrapped in unbleached muslin, which were secured around the neck of each sheep with hog-ring fasteners. The last group, designated Vapona principals, was fitted with Vapona collars made of the same material, except that the cotton webbing had been first soaked in 50% DDVP emulsifiable concentrate for 1 hour before being wrapped in the unbleached muslin. These were secured to the necks of each sheep in the last group in a similar manner. Each group was then held in separate 1.3-acre drylots.

Three days after treatment, the 10 sheep, designated dimethoate controls, were necropsied to determine whether or not the dimethoate therapy had been successful in eliminating all of the O. ovis larvae from their nasal passages and frontal sinuses. The examinations revealed 5 dead third instar larvae, one each in the frontal sinuses of 5 sheep.

About 8 months later, on March 30, 1966, at a time when the population of second and third instar larvae should be at its peak, the surviving sheep in the 2 remaining groups were necropsied and examined for O. ovis larvae.

A total of 10 firsts, 3 seconds, and 3 thirds was found in the 5 sheep in the Vapona principal group that survived the winter. Only one second instar was found in the 4 sheep in the Vapona control group.

This method does not appear to have promise of protecting sheep against the strike of the questing female nose bot fly. Also, the experimental design needs to be modified to include more sheep, kept under more suitable conditions for O. ovis activity.

(Albuquerque, New Mexico) (ADP b3-23)

H. Biology and Control of Psorergates ovis, the Australian Itch Mite of Sheep.

Since 1963, Psorergates ovis has been successfully maintained at the ADP laboratory in Albuquerque. From a single host acquired in that year, the infestation was naturally transmitted, through close contact in a small

pen, to 6 recipient crossbred southwestern range sheep during the subsequent 2 years. In May 1965, these 7 subjects were placed in a specially constructed, bird-proof cage, 20 by 40 feet, into which were introduced 19 additional sheep, including a 2-year-old Suffolk ram.

In December 1965, lambs began to drop, and by March 1966, a total of 17 had been born to the above flock; in January 1966, the ram was removed from the compound. As of June 1966, the infestation had transferred to 4 recipient ewes, and no lambs. Of the 7 original donors, only 4 retained detectable infestations.

This experiment demonstrates the slow rate at which psorergatic mites transfer within the flock. Lambs did not acquire demonstrable infestations after 3 to 5 months of exposure, and only 22 percent of the ewes became demonstrably infested after exposure of a full year. The terms "detectable" or "demonstrable," in reference to infestations are used with due consideration because, unless heavy infestations are present, the existence of Ps. ovis is extremely difficult to detect.

(Albuquerque, New Mexico) (ADP b3-24)

I. Investigations into the Pathobiology of Several Laboratory and Field Strains of Psoroptes ovis

Host-Parasite Interactions Between Sheep and the Common Scabies Mite, Psoroptes ovis. During the past 5 years at Albuquerque, sheep infested with Psoroptes ovis, when maintained singly in isolation pens, eventually lost their infestations, and recovered spontaneously from scabies. Of a total of 44 sheep involved in these studies, 40 recovered completely, but 4 currently retain surviving infestations. Recovery took place in a minimum of 2 months, and a maximum of 26½ months. Of the 4 isolated hosts on which infestations presently survive, 2 have harbored live mites for 8 months, 1 for 21 months, and 1 for approximately 27 months.

Infestations of virulent and avirulent strains of mites both survived for an average of approximately 10 months. In fact, survival of infestations for an average of 10 months applied during the 5-year period in which these observations were conducted and during the first half of 1966 as well.

An effort to explain this phenomenon of spontaneous recovery from P. ovis infestations suggests some manner of host resistance. However, hosts recently recovered from thriving infestations manifested no resistance to reinfestation. Therefore, it is suggested that host resistance is a localized reaction, presumably associated with the dermis.

Monthly and Seasonal Studies of the Population Components of P. ovis Infestations on Sheep. The phenomenon of summer latency, or dormancy, of Psoroptes ovis infestations on sheep (and on cattle as well) has long been recognized. Population pressures of scabies mites subside each summer, and may or may not rise again in the fall. The factors are not established that are associated with the summer season and responsible for the apparent recovery of the host from the disease. Studies at this laboratory on the periodic latency of scabies have, during recent years, resulted in interesting contributions. The present study, reported herein, was designed to determine the components of the mite population involved in sheep infestation that are capable of oversummering and hence responsible for perpetuation of the species.

The plan of work involves removal of all mites from a prescribed area of an active lesion each month during the year, breaking the population down into its 8 components, and showing variations in population components from month to month, or season to season, if any. To date, the study involves sampling of overall parasite populations during 8 months, beginning in November 1965. A total of approximately 4,000 eggs and 4,500 motile forms of mites has been examined in this manner.

An attempt was made to show variations in each of the motile population components, in relation to the entire population as collected monthly, on a percentage basis. The most profound fluctuation in population occurred in the larval stage, followed by the adult male stage. Segments of the population that fluctuated least, from month to month, were (1) male protonymphs, (2) female protonymphs, (3) deutonymphs, and (4) pubescent females. The ovigerous female segment of the population varied from a high of 30 percent to a low of 10 percent of the total population.

(Albuquerque, New Mexico) (ADP b3-25)

J. Overwinter Survival of Parasitic Nematode Larvae on Mississippi Pastures.

An experiment was conducted to determine whether infective nematode larvae overwinter on pasture in Mississippi. One-acre Bermuda grass pastures were used at each of the experiment stations at State College and Natchez. The plots were heavily grazed in 1964 by sheep infected with Haemonchus contortus, Trichostrongylus axei, Ostertagia circumcincta, Cooperia curticei, C. punctata, Trichostrongylus colubriformis, Strongyloides papillosus, Nematodirus spp., Trichuris ovis, and Oesophagostomum columbianum. When the sheep were removed from the pasture plots in August at State College and late September at Natchez, the plots were closed to any further grazing. In late April 1966, 5 three-month-old lambs raised worm-free and having negative fecal examinations were placed on the pasture plots and allowed to graze for 10 days. They were then placed in a concrete floored barn and held for 30 days before necropsy on May 21.

At both places, the grass in the plots had grown so high by the time the lambs were ready to go on the plots, the 5 lambs could not begin to sample completely the pasture in the 10 days allotted. A longer period of time was not used inasmuch as this would have allowed maturation of worms picked up and further contamination of the pastures.

Few worms were encountered at necropsy, but specimens of Haemonchus contortus, Strongyloides papillosus, Trichuris ovis, and Moneizia spp. were recovered.

(Auburn, Alabama and State College, Mississippi) (ADP b3-26)

K. The Biology and Control of the Liver Fluke, Fasciola hepatica, in the Southwest.

Liver fluke disease is widespread in sheep and cattle in high-rainfall areas of northern New Mexico, southern Colorado, and eastern Arizona. Drugs are now available that will remove the adult flukes; however, these same drugs have little or no effect in removing the young parasites. Treatment often is not successful from a clinical standpoint because a new population stemming from young flukes, which resist treatment, may grow up within a few weeks. Research is oriented toward finding chemical compounds that are effective against these young flukes. During the past year, two compounds - bithionol and sulfoxide of bithionol - were studied. Both compounds were very effective against the adults but were ineffective against the immature flukes.

(University Park, New Mexico) (ADP b3-27)

A single oral dose with 50 mg/kg of bithionol, tested on mature Fasciola gigantica in artificially infected sheep, was found 100 percent effective. Forty-eight hours after the treatment with 50 mg/kg or higher doses of the chemical, no living giant flukes were found at necropsy. No side effects, other than soft feces and diarrhea that lasted 24 hours, were observed in 1 sheep given 3 times the therapeutic dose. Bithionol had no adverse effect on the hatchability of F. gigantica eggs collected from the gall bladders of the sheep treated with 50, 60, and 100 mg/kg.

(Ankara, Turkey) (A22-ADP-1)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

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Coccidial Parasites

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PUBLICATIONS -- STATE EXPERIMENT STATIONS

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AREA NO. 14 -- PARASITES AND PARASITIC DISEASES OF POULTRY

Problem. Parasites and parasitic diseases probably cost the poultry industry many millions of dollars annually by causing intestinal disturbances, emaciation, retarded growth, reduced egg production, and deaths. Parasites are ubiquitous, many times insidious, and often overlooked until birds are damaged irreparably. Early diagnosis is difficult, and reliable treatments for many devastating parasitoses are not available. Moreover, some management practices, intended to avoid spread of parasites and to control them, have been found ineffectual as is shown by the increasing importance of certain parasites in broiler production. The problem is to develop, through a planned, balanced program of basic and applied research, methods for preventing, controlling, or eradicating parasitic diseases, thus affording economical production of healthy poultry and sound products in supplies adequate to meet the needs of an expanding population.

USDA AND COOPERATIVE PROGRAM

The Department has a continuous long-term program involving parasitologists, biologists, and chemists, engaged in both basic studies and the application of known principles to the solution of the problem of parasites and parasitic diseases of poultry.

The Federal scientific effort devoted to research in this area totals 4.0 scientific man-years. This effort is applied as follows:

Control of Coccidiosis 2.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Biology of Nematode Parasites 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Biological Investigations of Protozoan Parasites and Parasitic Diseases, with Special Reference to Those of the Gastrointestinal Tract 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 2.6 scientific man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Biological Investigations of Protozoan Parasites.

Histomonas meleagridis, the protozoan that causes blackhead in turkeys, chickens, and several species of game birds, has been grown in artificial mediums continuously for 6 years and involved more than 700 serial passages. By this time, the organism grew poorly in both chickens and turkeys, and produced no lesions whatsoever, apparently having lost its ability to invade the tissues. However, it still possessed enough immunizing ability to protect 20 to 50% of the birds that were given challenge exposure with virulent H. meleagridis 3 weeks after immunization. This immunizing ability declined rapidly between the 730th and 835th passages. There is some evidence that long-continued cultivation on artificial mediums operated selectively on the mixture of virulent and relatively avirulent strains that commonly make up a wild population. Apparently, the organisms best adapted to tissue invasion were gradually lost.

Japanese quail are frequently used for experimental work and are usually caged in buildings near other birds, such as chickens, turkeys, and game birds. The common cecal worm, Heterakis, and the protozoan that causes blackhead in chickens, turkeys, and some game birds could be troublesome parasites if they developed as contaminants in any of the caged birds, including the Japanese quail. Our studies showed that these small birds could be infected experimentally with both the cecal worm and the protozoan, Histomonas, but neither thrived well in the caged birds. It is doubtful that the Japanese quail, properly confined, would become a source of infection for either parasite in susceptible birds housed nearby, or cared for by the same attendants.

Several common species of earthworms were found to transmit to chickens and turkeys both the cecal worm, Heterakis, and Histomonas, the protozoan parasite that causes blackhead. As the earthworms feed, they take in the eggs of the cecal worm, and these eggs soon hatch. The young larvae leave the cavity of the digestive tract, entering the tissues or the coelomic cavities, where they sometimes accumulate in large numbers. When a chicken, turkey, or other suitable host consumes the earthworm in which the larvae have accumulated, the latter are liberated and enter the bird's ceca. If the larvae carry the protozoan, Histomonas, it may be liberated, and the bird may develop blackhead. Both the larval cecal worms and the histomonads survive in earthworms from one season to the next, although the numbers are fewer after the earthworm's hibernation.

(Beltsville, Maryland) (ADP b4-11)

In cooperative research at the Texas Agricultural Experiment Station, preliminary attempts failed to incriminate the darkling beetle as a transport host of Histomonas meleagridis. Histomonads were cultured in vitro in embryonated turkey eggs and in hamster cell culture systems. Serial passages were made in each system in the absence of bacteria and without the loss of infectivity to turkeys. Rabbits are being immunized with histomonad suspension in the hope that a conjugated antiserum can be made which will locate H. meleagridis in tissues.
(College Station, Texas) (ADP b4-11)

Under a PL 480 grant to the National Taiwan University, Taipei, Taiwan, China, studies on leucocytozoonosis of chickens are being conducted. The period from the end of April to September is the prevalent season of this disease in Taiwan. In order to decrease the losses of chickens caused by this protozoa, some therapeutic and preventive trials were performed in the first research year with the following results:

1) Seasonal outbreak of the disease began at the end of April, gradually extended to June and July, and then slowly decreased until the end of October. After October, the disease rarely occurred. In the prevalent season, the causative agent was Leucocytozoon caulleryi. L. sabrazesi and L. andrewsi were also found in the summer season. Furthermore, the latter two Leucocytozoon spp. were found in the winter season and caused some outbreaks, but less damage to poultry.

2) Therapeutic trials tested 5 drugs that had been reported effective in the treatment of this disease. Drugs tested were croran, antilicone, atabrin, antrycide, and furazolidone. These drugs were given to 36 experimental chickens that were infected artificially with L. caulleryi in stage I to III and stage IV to V of the gametogony. Although the drugs improved the systemic symptoms and decreased the mortality rate in affected chickens, they did not stem the disease in the prevalent season.

3) Preventive efficacies, side effects, and economic values of 7 drugs were analyzed. One hundred ninety five chickens were given 0.0025% sulfadimethoxine, 0.0025% sulfamethoxypyridazine, 0.015% sulfisomezole, 0.02% sulfamethazine, and 0.00005% pyrimethamine in their feed for 105 successive days. During this period, there was complete prevention of the disease without side effects, in spite of repeated inoculation of causative agents at 30-day intervals. Furazolidone, 0.01%, in feed had inhibitory action against the infection when fed continuously, but antrycide had none. Among the 5 drugs which control the infection, pyrimethamine and sulfamethoxypyridazine were the most economical. Sulfadimethoxine and sulfamethazine were also effective against fowl coccidiosis. Sulfisomezole was costly, and could not control fowl coccidiosis. From these results, poultry farmers could choose sulfadimethoxine, sulfamethoxypyridazine, sulfamethazine, and/or pyrimethamine to prevent fowl leucocytozoonosis according to their individual needs. Pyrimethamine was not strong enough for this infection.

Although no drugs were found to control the infection, the 5 drugs mentioned above were found to have satisfactory preventive effects, and are becoming extensively used in the poultry industry in Taiwan.
(Taipei, Taiwan, China) (A6-ADP-1)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Protozoan Parasites

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Augustine, P. C., and Ellis, D. J. 1966. Immunizing action of in vitro-attenuated Histomonas meleagridis in chickens and turkeys. Exp. Parasit. 18(3).

PUBLICATIONS -- STATE EXPERIMENT STATIONS

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Kemp, R. L., and Reid, W. M. 1965. Pathogenicity studies on Trichomonas gallinarum in domestic poultry. Poult. Sci. 44(1):215-221. Ga.

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Renault, L. W. M., Reid, M., and Johnson, J. 1965. Identification in France de la coccidiose intestinale aviaire provoquee par Eimeria brunetti. Rec. Med. Vet. 140:463-467. Ga.

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AREA NO. 15 - TREATMENT FOR REMOVAL OF PARASITES OF DOMESTIC ANIMALS

Problem. Parasites of food animals are responsible for losses to livestock producers approximating a billion dollars annually. This estimate, moreover, is conservative since it does not take into account costs of treatment and other control measures. Chemical antiparasitic agents are the most powerful weapons presently available against parasites and the diseases they cause, yet specific treatments generally have a comparatively short period of usefulness. Many of the currently preferred treatments were unknown a decade or so ago and, in all probability, few, if any, of those in use today will be primary choices a decade or so hence. Moreover, the growing concern with respect to residues in edible tissues and organs of treated animals and birds necessitates development of control measures other than treatment. The problem is to develop, through a planned, balanced program of basic and applied research, control methods that minimize reliance on extrinsic chemicals. These include investigations of immunological procedures, management practices which minimize exposure of animals to parasitic infections, and natural control agents such as parasites, pathogenic microorganisms, and predators of economically important livestock pests.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving veterinarians, parasitologists, pharmacologists, and biochemists engaged in both basic studies and the application of known principles in developing treatments for removal or control of parasites of domestic animals. Research is being conducted on this problem at the following designated locations.

The Federal scientific effort devoted to research in this area totals 9.5 scientific man-years. This effort is applied as follows:

New and Improved Anthelmintics 3.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Hazards of Residues from Treatment for Parasites 2.0 at the Regional Animal Disease Research Laboratory, Auburn, Alabama.

Evaluation and Standardization of Antiparasitics 3.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland, and at the Regional Animal Disease Research Laboratory, Auburn, Alabama.

Control of Lice on Cattle 1.5 at the Albuquerque, New Mexico, field station.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 9.1 scientific man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Control of Internal Parasites of Livestock by Management Practices That Will Not Create Consumer Residue Hazards.

At the Experiment, Georgia, substation of the Regional Research Laboratory, Auburn, Alabama, a study was completed on the epizootiology of helminth parasites of sheep in Georgia. September was the most critical month for grazing pastures, as evidenced by the largest number of eggs passed. Younger animals were more susceptible to all parasites than older ones, as judged from the egg counts.

The second year of a test to determine the effects of various management practices in the control of internal parasites of lambs was finished. Three lots were planted with a winter temporary pasture mixture and 2 lots with rescuegrass. Each type had a continuously grazed pasture and also a continuously grazed pasture with creep feeding of corn. One of the temporary pastures was grazed on a four-way rotational grazing system and the lambs were creep fed. The creep-fed lambs gained more weight and had higher slaughter and carcass grades than the lambs that did not receive grain. In general, although not statistically significant, more worms were recovered from lambs on a rotationally grazed winter pasture plus creep feeding (3,215) than those continuously grazed on comparable pasture plus creep (3,064). Lambs on rescuegrass generally exhibited more worms than those on temporary pasture. Also, less worms were obtained from the creep-fed lambs than from those on comparable pastures without supplemental corn. The average number of worms recovered from lambs on both rescuegrass pastures was greater than that from those on comparable winter temporary pastures.

(Experiment, Georgia) (ADP b5-16)

B. Investigations to Develop New and Improved Chemical Agents for the Treatment, Prevention, or Control of Helminthic Parasites in Farm Animals.

At the Beltsville Parasitological Laboratory, researchers found that Haloxon (0,0-di(2 chloroethyl)-0-(3-chloro-4-methylcoumarin-7-yl) phosphate) had marked anthelmintic action against the cropworm, Capillaria contorta, of quail (Colinus virginianus). Best results were obtained when the chemical was given at concentrations of 0.075 and 0.1% of the regular ration for periods of 5 to 7 days. At these levels, the drug was well tolerated and removed from 92 to 100% of the worms. Excellent results were also obtained when Haloxon was given at the 0.5% level for 5 days, but all treated birds developed incoordination and 3 died. Single oral doses of the drug, given at the rate of 25 or 50 mg./kg. body weight, were not uniformly

effective and elicited undesirable side effects, primarily ataxia.

Methyridine (2-(beta-methoxyethyl) pyridine) showed little promise as a practical chemotherapeutic agent for use against C. contorta of quail. The drug was given by subcutaneous injection in single or multiple doses ranging from 11 to 90 mg./bird. Larger doses produced significant anthelmintic action. But signs of toxicity, including death, were noted in several test groups. Excellent anthelmintic action was also obtained with regimens involving 3 injections of 35 or 45 mg. at 4- to 5-day intervals, but the procedures would have limited applicability for general use.

Haloxon was completely effective against the large intestinal roundworm, Toxocara canis, of dogs when given in single doses of 50, 100, and 200 mg./kg. of body weight. At these levels, the drug removed a total of 74 roundworms from 5 dogs; an additional dog that received the 200-mg. dosage was not infected with Toxocara. Haloxon showed no significant action against whipworms, the only other helminth harbored by the test animals.

(Beltsville, Maryland) (ADP b5-18)

C. Investigations of Potential Chemotherapeutic Agents as Treatments for Bovine Venereal Trichomoniasis.

In research work at the Beltsville Parasitological Laboratory 9 bulls were freed of venereal trichomoniasis by single or multiple intravenous injections of dimetridazole.

Drug resistance was experimentally induced in strains of Tritrichomonas foetus established in the vagina of hamsters. These laboratory strains were also cross resistant to other compounds with trichomonacidal properties.

Concomitant treatment with quinacrine hydrochloride did not prevent Tritrichomonas foetus experimentally established in hamsters from developing a tolerance for dimetridazole when the latter chemical was used at sub-optimal levels.

(Beltsville, Maryland) (ADP b5-19)

D. Evaluation, Development, and Standardization of Antiparasitics.

Researchers at the Beltsville Parasitological Laboratory report that strains of Eimeria tenella developed a tolerance for Unistat and arsensobenzene as a result of repeated passage through chickens fed mash containing the coccidiostats. The Unistat-resistant strain is cross resistant to 3 other coccidiostats and the arsenosobenzene-resistant strain to 5 others.

A strain of E. tenella developed a pronounced tolerance for amprolium as the result of serial propagation in groups of chickens fed this coccidiostat.

The addition of quinacrine hydrochloride to mash medicated with glycarbylamide and fed to groups of chickens through which a strain of E. tenella was serially propagated did not prevent the development of resistance to glycarbylamide in the coccidial strain.

Novastat, a recently introduced coccidiostat, effectively controlled experimental E. tenella infections in chickens.

Unsporulated oocysts of E. tenella that had been stored at refrigerator temperature for 5 months failed to develop to the infective stage when returned to room temperature.

Dithiazanine iodide was totally effective against experimentally induced infections of the common hookworm, Ancylostoma caninum, in dogs.

The drug was given in feed daily for 14 days at a dose rate of 25 mg./kg. of body weight. Other than staining of the feces, there were no unusual effects from treatment with this cyanine dye.

In controlled anthelmintic tests with lambs, thiabendazole had marked anthelmintic action against experimentally induced infections of the common stomach worm, Haemonchus contortus. The drug was given from 1 to 4 weeks after inoculation with infective larvae and in dosages of 50 and 100 mg./kg. of body weight. Comparative necropsy worm count data between principals and untreated controls showed that the drug removed at least 97% of the worms at both dosage levels when given 2 or more weeks after inoculation. Moreover, thiabendazole, at a dose rate of 50 mg./kg., was about 92% effective against 1-week-old infections.

(Beltsville, Maryland) (ADP b5-20)

The Regional Animal Disease Laboratory, Auburn, Alabama, reported as follows on studies conducted at State College, Mississippi.

Continuous feeding of a low level of phenothiazine for 122 days to heifers in drylot, or by means of a therapeutic drench or a combination of feeding and drenching, had no beneficial effects on average daily gains. This lack of beneficial effect could probably be explained by the fact that the number of worms found at necropsy and by egg counts was below that which would produce weight loss. Worm egg counts decreased as the test progressed, possibly as a result of aging of worms with no opportunity for reinfection in drylot. Counts of larvae at the end of the test were lower than ones made at the beginning though there was little difference in numbers among the various treatments.

The low level feeding of phenothiazine appeared to reduce the numbers of the large stomach worm (Haemonchus placei), hookworms (Bunostomum phlebotomum), nodular worms (Oesophagostomum radiatum), and cooperids

(Cooperia oncophora); however, the differences in numbers of worms were not statistically significant at the 5% level of probability.

(State College, Mississippi) (ADP b5-20)

At Experiment Georgia, substation of the Regional Animal Disease Laboratory, Auburn, Alabama three formulations of Maretin (0,0-diethyl-0-(naphthaloximido) phosphate) were tested for effectiveness in the removal of gastrointestinal nematodes from naturally-infected ewes in Georgia. At a dose level of 50 mg./kg. of body weight, all treatments effectively reduced the number of eggs of the Cooperia-Trichostrongylus-Ostertagia group and of Haemonchus spp. There was no evidence of toxicity in any of the treated ewes.

(Experiment, Georgia) (ADP b5-20)

E. Control of Lice on Cattle.

The Regional Research Laboratory, Albuquerque, New Mexico investigated new approaches to the chemical control of lice on cattle.

Famphur, an organophosphate compound known to be highly effective as a systemic against cattle grubs, was effective against the short-nosed cattle louse when administered as a feed additive. The material was given orally, in the feed, at the rate of 2.5 mg./kg. of body weight; after 30 days of this regimen, all short-nosed, blood sucking lice were destroyed, and louse populations on the most heavily infested cattle were eliminated.

A simple method of controlling cattle grubs, now in vogue, consists of pouring small quantities of highly concentrated systemic drug formulations on the backs of cattle. The material is then translocated deep within the body, destroying migrating cattle grubs wherever they may be located. One of these "pour-ons" (famphur, 16.5% in oil solution) has been effective against the blood sucking, short-nosed cattle louse, Haematopinus eurysternus. When poured on the backs of cattle at the rate of 210 mg./kg. of body weight, a single application of famphur destroyed all short-nosed lice, and eliminated infestations completely from heavily infested animals.

Neck bands containing dichlorvos, applied to cattle, eliminated heavy infestations of the short-nosed cattle louse and the little red chewing louse. Both slow-acting commercial resin strips (5% dichlorvos) and laboratory-prepared nylon webbing containing DDVP (43% dichlorvos) were effective. Lice were eliminated in approximately 1 month to 10 weeks, depending upon the amount of drug incorporated in the band.

(Albuquerque, New Mexico) (ADP b5-21)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

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AREA 16 - MISCELLANEOUS PARASITES AND PARASITIC DISEASES

Problem. Parasitism is a way of life that characterizes the majority of living things. Except for basic life processes, it is probably the commonest biological phenomenon. More than 50,000 kinds of animal parasites (i.e., parasites classified as animals as opposed to those classified as plants) are known. New varieties are being discovered and described at a rate of about 500 per year. Some devastating parasites, indigenous to foreign countries, threaten to surmount barriers imposed against them. Certain of these have already gained new footholds in livestock, poultry, and wildlife. Essential elements of procedure against parasites--established, exotic, or new--are accurate diagnosis, development of full knowledge about them, and research on effective control measures. The primary requirement is development through research of up-to-date knowledge of classification and identification supported by a complete reference collection of parasites, including type specimens and familiarity with global research already done. Basic investigations of parasitisms as biological phenomena are involved, especially in host-parasite relations, immunology, serology, ultrastructure, and other aspects of diagnosis and control. The problem is to develop and maintain up-to-date methods of identification and the essential, supporting reference collections, as well as complete parasitological information extracted from the world's scientific literature; investigate important phenomena and host-parasite systems not covered in specific host categories; and provide bases for detection and control that are adequate to meet existing and anticipated needs, through research on problems involving various parasites and hosts, including wild animals and birds important to agriculture.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program for parasitologists, biochemists, microbiologists, and veterinarians engaged in basic and applied research in this area. Research is being conducted on the following problems at the designated locations.

The Federal scientific effort devoted to research in this area totals 11.5 scientific man-years. This effort is divided among subheadings as follows:

Publication and Maintenance of Author, Subject, and Host Index-Catalogues 2.0 at the Beltsville Parasitological Laboratory.

Immunologic and Other Biologic Approaches to the Prevention and Control of Parasitic Diseases 1.7 at the Beltsville Parasitological Laboratory and under cooperative agreement with the College of Veterinary Medicine, University of Minnesota, St. Paul, and Wisconsin Agricultural Experiment Station, Madison.

Chemical and Physical Elements of Parasites and Parasite-Host Relationship 2.0 at the Beltsville Parasitological Laboratory.

Taxonomic Investigations and Identification of Parasites 2.0 at the Beltsville Parasitological Laboratory.

Maintenance of Parasite Collection 0.3 at the Beltsville Parasitological Laboratory.

Pigments of Parasites 0.5 at the Beltsville Parasitological Laboratory.

Biology, Epidemiology, and Pathogenicity of Demodectic Mange 1.0 at the Parasite Research Laboratory, Albuquerque, New Mexico.

Cytological Investigations of Protozoan Parasites That Penetrate the Gastro-intestinal Tract of Poultry and Other Farm Animals 2.0 at the Beltsville Parasitological Laboratory.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 3.4 scientific man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Publication and Maintenance of Author, Parasite-Subject, and Host Catalogues of the Index-Catalogue of Medical and Veterinary Zoology.

Material for the Parasite-Subject, Treatment, and Host Sections of Supplement 16, the Author Section of Supplement 17, and the Subjects: Trematoda and Trematode Diseases, Part 6, Supergenera and Genera N-P, is being prepared for publication. The Host Section of Supplement 15, the Authors: A-Z and the Protozoa part of Parasite-Subject Section of Supplement 16 are in press. The Parasite Section (Protozoa, Trematoda and Cestoda, Nematoda and Acanthocephala, Arthropoda, etc.) and Subject-Headings and Treatment Sections of Supplement 15 were published in the spring of 1966. The Subjects: Trematoda and Trematode Diseases, Part 4, Supergenera and Genera E-G, was published in the fall of 1965, and Part 5, H-M, was published in the spring of 1966. (Beltsville, Maryland)(ADP b6-9(Rev.)

The Index-Catalogue of Medical and Veterinary Zoology has been maintained and expanded in its various sections: Author, Parasite-Subject, and Host Catalogues, and Checklist of Specific and Subspecific Names. New entries augmenting the Catalogues are as follows: Author entries, 4,658, Parasite-Subject entries, 38,566 (including 25,248 parasite entries, 1,979 anthelmintic entries, 2,349 subject heading entries); and Host entries, 8,990.

New genera and species of parasites are as follows: Protozoa: 16 n.g., 66 n.sp.; Trematoda: 36 n.g., 239 n.sp.; Cestoda: 16 n.g., 80 n.sp.; Nematoda: 52 n.g., 381 n.sp.; Arthropoda and miscellaneous groups: 30 n.g., 443 n.sp. There have been 115 new citations of periodicals added to the Catalogue.

Monthly literature searches of approximately 20 different bibliographic journals were completed and unavailable material requested for indexing. Three bibliographies from the Medical Literature Analysis and Retrieval System (MEDLARS) have been received from National Library of Medicine, covering current parasitological literature; these and other material are being indexed. An average of 500 incoming periodicals were examined each day at the National Agricultural Library for parasitological papers.

The Index-Catalogue has had more than 70 visitors from the United States and five other countries, some of them staying several days and consulting the Catalogue as a source of information. (Beltsville, Maryland) (ADP b6-14)

B. Immunologic and Other Biologic Approaches to the Prevention and Control of Parasitic Diseases.

At the Beltsville Parasitological Laboratory, primary cell cultures of swine liver and kidney cells with a fluid medium composed of NCTC 109, serum, yeast extract, peptone, and glucose were used to obtain development of Stephanurus dentatus to late 4th stage. Yields, survival and advanced development were superior to that obtained in systems previously reported. (Beltsville, Maryland) (ADP b6-10)

During the past year, progress has been slow on the initiation of studies on the biological control of helminth parasites of cattle, sheep and poultry. Extensive review of the literature has been in progress and work has been started on the large American liver fluke (Fascioloides magna) and on the host parasite relationships of the cecal worm (Heterakis gallinae) to blackhead (histomoniasis) of turkeys.

Potential snail intermediate hosts have been collected from areas where fluke infection is a problem. These mollusks have been maintained in the laboratory to provide a snail supply that can be infected with F. magna and to provide a source of supply of infective stages of the liver fluke for use in future experimentation.

The studies on blackhead and the cecal worm have been directed toward attempts to establish pure cultures of the blackhead organism (Histomonas meleagridis) and the cecal worm (Heterakis gallinae) in the young turkey and chicken.

In general, it is proposed to investigate both of these host-parasite relationships from a biological, physiological and immunological aspect. When a suitable supply of infective material is available, it is hoped to study the host responses to irradiated forms of the liver fluke, the blackhead organism and the cecal worm. (St. Paul, Minnesota) (ADP b6-10)

The first emphasis has been given to the collection of Haemonchus populations in isolated environmental areas of the U.S. Twenty-six collections have been made in the northwest U.S., and the Haemonchus populations cultured for study in Wisconsin. At the present time, 7 populations are in controlled critical experiments designed to compare relative pathogenicity and 11 other populations are being processed in "seed lambs."

Following the critical comparisons, the less pathogenic populations will be retained, and further collections from Wisconsin and from the northeast U.S. will be introduced into the project for further comparisons of variation in pathogenicity. (Madison, Wisconsin)(ADP b6-10)

C. Chemical and Physical Elements of Parasites and Parasite-Host-Relationships.

Zone and barrier electrophoresis through agar medium of mixtures of kidney worm (Stephanuras dentatus) antigens yielded antigen fractions of improved specificity as compared to the original mixtures. The fraction antigens detected antibodies in sera earlier in the course of infection than did the mixed control antigens and gave fewer reactions with normal sera.
(Beltsville, Maryland)(ADP b6-11)

D. Taxonomic Investigations and Identification of Helminths and Other Parasites.

A study of the classification, morphology, hosts, and distribution of the filariid worms of canines of the world was submitted for publication as a chapter in a book on canine filariasis. Numerous illustrations and tables on the diagnostic characteristics of the various species of Dirofilaria, Cystofilaria, Dipetalonema, and Brugia that infect canines were included in the manuscript. A study was completed on the parasites of the mountain sheep, Ovis canadensis, of North America. Specimens were examined in the Animal Parasite Collection and from 18 mountain sheep necropsied in three localities in Montana. The findings were summarized in a manuscript with information from published reports on the distribution of all parasites of this host. This study has increased from 34 to 51 the known parasites that occur in/on mountain sheep. Seventy percent of this number are known parasites of domestic sheep and 35 percent of cattle in North America. An unexpected result of the study was the finding that species of gastrointestinal nematodes of mountain sheep can vary greatly between localities

within a state. Species recovered from two separate mountain sheep populations in Montana, within 40 miles of one another, were totally different. Among the nematodes examined from the Montana mountain sheep were found specimens of a thread-necked strongyle, Nematodirus davtiani Grigorian, 1949, not known to occur in North America. This nematode was described from specimens recovered from a wild sheep in Russia and it has been reported from domestic sheep and goats in that country. A comparison of the description of N. davtiani with the type specimens of Nematodirus rufaevastitatis Durbin and Honess, 1951, described from domestic sheep in Wyoming, indicated that the two species are conspecific. A manuscript has been accepted for publication which suppresses the name N. rufaevastitatis as a synonym of N. davtiani. Hence, N. davtiani is also a parasite of domestic sheep in this country. (Beltsville, Maryland) (ADP b6-12)

One hundred and thirty-one lots of specimens were identified (trematodes 10, cestodes 8, acanthocephalans 3, nematodes 37, and arthropods 73). Among these were numerous parasites of medical and veterinary importance. A large number of Onchocerca lienalis collected from cattle at abattoirs at St. Paul, Minnesota, were identified. These specimens indicate that O. lienalis is a common parasite of cattle in the midwest, where previously it has only been known to occur in Missouri and Illinois. Other specimens identified include Stephanurus dentatus in pork received from Kentucky and Virginia, ascarid fragments in a porkchop, and a larval ascarid in the brain of a hedgehog. The following ticks were collected from animals offered for entry into the United States: Amblyomma cajennense, A. gemma, A. pomposum, A. tholloni, A. variegatum, Boophilus sp., Dermacentor nigrolineatus, D. nitens, Ixodes hexagnonus, Ornithodoros megnini, Rhipicephalus pulchellus, and R. simus. Ninety inquiries concerning the identification of parasites were answered during the year. (Beltsville, Maryland) (ADP b6-16)

E. Maintenance of Parasite Collection

Eight hundred and five lots of specimens (protozoans 3, trematodes 162, cestodes 88, acanthocephalans 36, nematodes 414, arthropods 99, and miscellaneous 3) were added to the parasite collection. These include many type specimens of new genera and species. Thousands of specimens in bottles were examined and several hundred bottles required additional preserving fluid and new covers. One hundred and forty-one inquiries concerning the deposit or loan of specimens were answered during the year.
(Beltsville, Maryland)(ADP b6-15)

F. Pigments of Parasites

phoretic component; the hemoglobin of the host contained two. The single component in parasite hemoglobin migrated faster than either of the two components of the host hemoglobin.

The turkey hemoglobin had a lower sedimentation coefficient than the turkey hemoglobin. The worm pigment was completely denatured in less than one minute in alkali (pH 12.5), but the turkey hemoglobin was not completely denatured even in 15 minutes.

In summary, gapeworm hemoglobin is not turkey hemoglobin: the two pigments differ in several physico-chemical properties. (Beltsville, Maryland)
(ADP b6-17)

G. Biology, Epidemiology, and Pathogenicity of Demodectic Mange of Domestic Livestock.

The study of demodectic mite parasitism of cattle was initiated as a project at the ADP laboratory, Albuquerque, New Mexico in June, 1965. For many years the hide and tanning industry has reported considerable losses from the damage produced by this parasite in leather from the hides of infested cattle, swine, goats, and possibly horses.

Despite the fact that published reports on the demodectic mite parasitism of domesticated and wild animals, as well as man, date back to the 1840's, the mode of exposure of susceptible animals to this arachnid parasitism has not been elucidated. Until reliable factual information is available, no practical means of the prevention and control of demodecosis in man and animals can be developed.

During the past fiscal year, documented reports were received at intervals which indicate the serious economic aspects and the general distribution of this bovine parasitism in the United States. Surveys of cattle in New Mexico and nearby regions suggested that very few cases exist in native beef cattle. The local dairy animals which were verified clinical cases were imports, the progeny of animals introduced from the eastern and western regions, or they were from areas where there was an active interchange with imported animals. There might be some benefit from this interesting environmental phenomenon because our native animals serve as good controls.

During the year, the following parasitized animals were purchased from neighboring states: a yearling grade Hereford heifer raised in Texas; a mature female dairy goat from a dairy herd in El Paso, Texas; and a mature (2-year-old) purebred Corriedale ram from a flock in Enid, Oklahoma. As far as is known, the Corriedale ram is the only verified clinical case of

demodectic mange existing at this time in sheep in the United States. (Albuquerque, New Mexico)(ADP b6-18)

H. Cytological Investigations of Protozoan Parasites that Penetrate the Gastrointestinal Tract of Poultry and Other Farm Animals.

Eimeria meleagrimitis, a coccidium parasitizing the small intestine of turkeys, completed 2 asexual generations in monolayer cultures of secondary bovine kidney cells. They did not develop in primary cultures of bovine kidney cells or in diploid swine kidney cells. The time intervals of development were the same as in vivo, but the number of merozoites (16-32) was far less than when found in the host (80-100).

Eimeria acervulina and E. necatrix, two species parasitizing chickens, developed only to the 4-nucleate schizont stage in the same type cultures in which E. meleagrimitis completed two generations.

The life cycle of Eimeria acervulina, a common coccidium parasitizing the duodenum of chickens, consists of 4 asexual generations. Schizonts of the 1st generation occur in the cells lining the glands of Lieberkühn and take 36-48 hrs. to develop. Those of the 2nd generation occur in the necks of the glands, those of the 3rd generation at the base of the villi, and those of the 4th generation at the sides and tips of the villi. The entire endogenous cycle of schizogony and gametogony takes 96 hrs. for completion.

PUBLICATIONS - USDA AND COOPERATIVE PROGRAMS

Publication of Index-Catalogues

Doss, Mildred A., with the assistance of Roach, Katharine F., and Breen, Virginia L. 1966. Index-Catalogue of Medical and Veterinary Zoology. Subjects: Trematoda and Trematode Diseases. Part 4, Supergenera and Genera E-G. U. S. Government Printing Office.

_____, with the assistance of Roach, Katharine F., and Breen, Virginia L. 1966. Index-Catalogue of Medical and Veterinary Zoology. Subjects: Trematoda and Trematode Diseases. Part 5, Supergenera and Genera H-M. U. S. Government Printing Office.

Humphrey, Judith M., and Segal, Dorothy B., with the assistance of Beard, Mary I., Edwards, Shirley J., and Kirby, Margie D. 1966. Index-Catalogue of Medical and Veterinary Zoology, Supplement 15, Parasite-Subject Catalogue. Subject-Headings - Treatment. U. S. Government Printing Office.

_____ and Segal, Dorothy B., with the assistance of Beard, Mary I., Edwards, Shirley J., and Kirby, Margie D. 1966. Index-Catalogue of Medical and Veterinary Zoology, Supplement 15, Parasite-Subject Catalogue. Subject-Headings - Treatment. U. S. Government Printing Office.

_____ and _____, with the assistance of Beard, Mary I., Edwards, Shirley J., and Kirby, Margie D. 1966. Index-Catalogue of Medical and Veterinary Zoology, Supplement 15, Parasite-Subject Catalogue. Parasites: Arthropoda, Mesozoa, Coelenterata, Mollusca, and Annelida. U. S. Government Printing Office.

_____ and _____, with the assistance of Beard, Mary I., Edwards, Shirley J., and Kirby, Margie D. 1966. Index- Catalogue of Medical and Veterinary Zoology, Supplement 15, Parasite-Subject Catalogue. Parasites: Trematoda and Cestoda. U. S. Government Printing Office.

_____ and _____, with the assistance of Beard, Mary I., Edwards, Shirley J., and Kirby, Margie D. 1966. Index-Catalogue of Medical and Veterinary Zoology, Supplement 15, Parasite-Subject Catalogue. Parasites: Nematoda and Acanthocephala. U. S. Government Printing Office.

Miscellaneous Parasitic Studies

Becklund, W. W. 1965. Nematodirus maculosus sp. n. (Nematoda: Trichostrongylidae) from the mountain goat, Oreamnos americanus, in North America. J. Parasit. 51:945-947.

Chitwood, M. B., and Jordan, H. E. 1965. Monodontus louisianensis sp. n. (Nematoda: Ancylostomatidae) a hookworm from the white-tailed deer, Odocolleus viroinianus (Zimmermann), and a key to the species of Monodontus. J. Parasit. 51:942-944.

_____ and Campillo, M. C. 1966. Texicospirura turki gen. et sp. n. (Nematoda: Spiruroidea) from the stomach of the peccary in the United States, and a key to the genera of Ascarosinae. J. Parasit. 52:307-310.

Doran, D. J. 1966. The migration of Eimeria acervulina sporozoites to the duodenal glands of Lieberkühn. J. Protozool. 13(1):27-33.

_____. 1966. Pancreatic enzymes initiating exceptation of Eimeria acervulina sporozoites. Proc. Helm. Soc. Wash. 33(1):42-43.

_____. 1966. Location and time of penetration of duodenal epithelial cells by Eimeria acervulina sporozoites. Proc. Helm. Soc. Wash. 33(1): 43-46.

PUBLICATIONS - STATE EXPERIMENT STATIONS

Alicata, J. E. 1965. Biology and distribution of the rat lungworm, Angiostrongylus cantonensis, and its relation to eosinophilic meningo-encephalitis and other neurological disorders of man and animals. Advances of Parasit. 3:223-248. Academic Press. Hawaii

Collins, R. C., and Dewhirst, L. W. 1965. Some effects of the suckling louse, Haemotopinus eurysternus, on cattle on unsupplemented range. J.A.V.M.A. 146(2):129-132. Ariz.

Hansen, M. F., et al. 1965. The black-tailed jack rabbit in Kansas. Pt. II. Helminth and arthropod parasites. KSU Agr. Experi. Sta. Bull. 140:38-64. Kan.

Harkema, Reinard, and Miller, Grover C. 1965. Notes on the life history of Strigea elegans, Chandler and Rausch 1947 (Trematoda:Strigeidae). J. Parasit. 51:894-895. N. Car.

Line Project Check List - Reporting Year July 1, 1965 to June 30, 1966

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Inc. in Summary of Progress (Yes-No)	Area and Subheading
ADP al	Infectious and Noninfectious Diseases of Cattle			
ADP al-9 (Rev. 2)	Diagnosis of bovine vibriosis by immunofluorescent methods **	Ames, Iowa	No	
		Ithaca, N. Y.	Yes	1-A
ADP al-13 (Rev.)	Tuberculosis of cattle	Ames, Iowa	Yes	1-B
		East Lansing, Mich. *	Yes	1-B
ADP al-14 (C)(Rev.)	Mucosal-respiratory disease-complex of cattle	Ames, Iowa	Yes	1-C
	Bovine virus diarrhea	Ames, Iowa	Yes	1-C
ADP al-15 (Rev.)	Mastitis of cattle	Ames, Iowa	Yes	1-D
		Davis, Calif.	Yes	1-D
ADP al-21 (Rev.)	Epizootic bovine abortion	Ames, Iowa	No	
		Davis, Calif.	Yes	1-E
ADP al-22	Investigations of foot rot (infectious pododermatitis) of cattle	Ames, Iowa	Yes	1-F
ADP al-24	Etiologic, cytologic, and histochemic studies of pulmonary adenomatosis in cattle	Ames, Iowa	Yes	1-G
ADP al-25	Immunization against bovine leptospirosis	Ames, Iowa	Yes	1-H
ADP al-26	Chemotherapy in leptospirosis disease of cattle and swine	Ames, Iowa	Yes	1-H
ADP al-28	Physiopathologic aspects of Lupinus sericeus and Lupinus caudatus plants on livestock **	Logan, Utah	Yes	1-N
ADP al-29 (C)	Enteritis in young calves	Moscow, Idaho	Yes	1-I
ADP al-30	Bovine lymphosarcoma	Ames, Iowa	Yes	1-J
		Lincoln, Neb.	Yes	1-J
		Ithaca, N. Y.	Yes	1-J
ADP al-31	Characterization of factors affecting the proliferation of Pasteurella sp. in the host	Ames, Iowa **	Yes	1-K
ADP al-32	Characterization and classification of members of the genus Brucella	Ames, Iowa	Yes	1-L
		St. Paul, Minn.	Yes	1-L
		Wooster, Ohio	Yes	1-L
		Madison, Wisc.	Yes	1-L
ADP al-33	The effect of Mycoplasma on bovine reproduction **	Ames, Iowa	No	
ADP al-35	Paratuberculosis (Johne's disease) of cattle	Ames, Iowa	Yes	1-M
ADP al-37	Pink-eye (Infectious Keratitis) of cattle	Ames, Iowa	No	
ADP al-38 (C)(Coop.)	Investigations to develop in vitro cytotoxic procedures for study and detection of tuberculosis sensitivity in cattle	East Lansing, Mich.	No	
ADP al-39	Pathogenesis of Pasteurella pneumonia **	Ames, Iowa	No	
ADP al-40	Immunogenic and bacteriologic studies of Vibrio fetus in cattle **	Ames, Iowa	Yes	1-A
PL 480 (A10-ADP-6)	The immunizing effect of brucella cell wall	Jerusalem, Israel	Yes	1-L

* Discontinued during reporting period.
** Initiated during reporting period.

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Summary of Progress (Yes-No)	Inc. in Area and Subheading
ADP a2	Infectious and Noninfectious Diseases of Swine			
ADP a2-13 (Rev.)	Pilot field studies to evaluate diagnostic tests, biologic products, and quarantine measures for a hog cholera eradication program	Ames, Iowa	Yes	2-A
ADP a2-15 (Rev.)	Erysipelas of swine	Ames, Iowa	Yes	2-B
ADP a2-16 (Rev.)	Brucellosis of swine	Ames, Iowa	Yes	2-C
ADP a2-17(C)	Hog Cholera	Ames, Iowa	Yes	2-A
		Lincoln, Nebraska	Yes	2-A-2
ADP a2-18	Infectious causes of infertility in swine other than brucellosis and leptospirosis	Ames, Iowa	No	
ADP a2-19	Abscesses in swine	Ames, Iowa	Yes	2-D
ADP a2-20	Etiology of atrophic rhinitis in swine *	Ames, Iowa	Yes	2-E
ADP a2-21	Pathogenesis of swine erysipelas *	Ames, Iowa	Yes	2-B
ADP a2-22	Effect of antiviral drugs on viruses associated with transmissible gastroenteritis (TGE) *	Ames, Iowa	Yes	2-F
ADP a2-23	Characterization of viruses associated with transmissible gastroenteritis (TGE) *	Davis, California	Yes	2-F
		Lafayette, Indiana	Yes	2-F

* Initiated during reporting period.

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Summary of Progress (Yes-No)	Inc. in Area and Subheading
ADP a3	Infectious and Noninfectious Diseases of Sheep and Goats			
ADP a3-4	Viral ulcerative dermatosis of sheep	Fort Collins, Colo.	Yes	3-E
ADP a3-5	Bluetongue in sheep - diagnosis, transmission and control	Denver, Colo.	Yes	3-A
ADP a3-6	Paratuberculosis (Johne's Disease) of sheep and goats	Ames, Iowa	Yes	3-D
ADP a3-7	Toxicological effects of oxalate-containing plants	Logan, Utah	Yes	3-F
ADP a3-8	Identification of teratogenic agent in Veratrum californicum	Logan, Utah	Yes	3-G
ADP a3-9	Chronic toxicity of herbicide accumulation in sheep tissues	Logan, Utah	Yes	3-H
ADP a3-10	Persistence and transmission of viral and rickettsial diseases in helminths,	Pullman, Washington	Yes	6-C
ADP a3-11	Metabolic, antigenic, and pathogenic characteristics of Vibrio fetus.*	Ames, Iowa	No	
		Fort Collins, Colo.	Yes	3-B
		Bozeman, Montana	Yes	3-B
		Logan, Utah	Yes	3-B
ADP a3-12	Immunology of scrapie *	Greenport, L. I., New York	Yes	3-C
PL 480				
E29-ADP-4	Etiological factors of scrapie in sheep	Edinburgh, Scotland	Yes	3-C
E29-ADP-5	Investigation of scrapie, a transmissible disease of sheep of obscure etiology	Compton, England	Yes	3-C

* Initiated during reporting period.

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Inc. in Summary of Progress (Yes-No)	Area and Subheading
ADP a4	Diseases and Parasites of Horses			
ADP b6-13(C)	Investigations on the serological diagnosis, transmission, and control of equine piroplasmosis	Beltsville, Maryland	Yes	4-A
		Gainesville, Florida	Yes	4-A
		Lexington, Kentucky	Yes	4-A
PL 480				
A22-ADP-4	Gastrophilus pseudo-hemorrhoidalis (equine parasite)	Ankara, Turkey	Yes	4-A
A22-ADP-7	Study of the horsesickness virus	Ankara, Turkey	Yes	4-A

Line Project Check List - Reporting Year July 1, 1965 to June 30, 1966

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Inc. in Summary of Progress (Yes-No)	Area and Subheading
ADP a5	Investigations of Infectious and Noninfectious Diseases of Poultry			
ADP a5-30, Supersedes ADP a5-2 (Rev.)	Salmonellosis of poultry	Athens, Ga.	Yes	5-B
ADP a7-25	Investigations of the genus Pasteurella	Ames, Iowa	Yes	5-C
ADP a5-29, Supersedes ADP a5-17	Chronic respiratory disease-complex in chickens and turkeys	Athens, Ga.	Yes	5-A
		State College, Miss.	Yes	5-A
		Raleigh, N. C.	Yes	5-A
		Jerusalem, Israel (A10-ADP-8)	Yes	5-A
ADP a5-28, Supersedes ADP a5-18	Newcastle disease	Athens, Ga.	Yes	5-D
		Ames, Iowa	Yes	5-D
		Orono, Maine	Yes	5-D
		Madison, Wisc.	Yes	5-D
		Pulawy, Poland (E21-ADP-6)	Yes	5-D
		(E21-ADP-7)	Yes	5-D
ADP a5-20	Ornithosis in poultry	Ames, Iowa	Yes	7-O
ADP a5-21	Turkey airsacculitis	Ames, Iowa	No	
		St. Paul, Minn.	Yes	5-A
		Madison, Wisc.	Yes	5-A
ADP a5-23	Infectious bronchitis in poultry	Athens, Ga.	Yes	5-E
		Ames, Iowa	Yes	5-E

Line Project Check List - Reporting Year July 1, 1965 to June 30, 1966

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Inc. in Summary of Progress (Yes-No)	Area and Subheading
ADP a6	Infectious and Noninfectious Diseases of Fur Animals			
ADP a6-5	Enteric disease complex of rabbits	Pullman, Washington	No	
ADP a6-6	Respiratory disease complex of rabbits	Pullman, Washington	No	
ADP a6-7	Field and laboratory studies of diseases of fur animals	Pullman, Washington Madison, Wisconsin	Yes	6-A
(ADP a3-10)	(Studies on the persistence and transmission of viral and rickettsial diseases in helminths)	(Pullman, Washington)	(Yes)	(6-B)

Line Project Check List - Reporting Year July 1, 1965 to June 30, 1966

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Summary of Progress (Yes-No)	Inc. in Area and Subheading
ADP a7	Miscellaneous Infectious and Non-infectious Diseases of Animals			
ADP a7-14 (Rev.)	Fractionation, purification, and characterization of the components of normal and immune sera of animals	Ames, Iowa	Yes	7-A
ADP a7-16 (Rev.)	Preparedness for laboratory assistance in diagnosis of foreign animal diseases	Greenport, Long Island, New York	Yes	7-B
ADP a7-17 (Rev.)	Studies to develop alleviators and diagnostic tests for plant poisoning and methods to avoid harmful residues in animal tissues from ingesting chemically treated plants	Logan, Utah	Yes	7-C
ADP a7-18 (Rev.)	Investigations in livestock of the biochemical effects of agricultural chemicals and control substances	Kerrville, Texas Nacogdoches, Texas	Yes Yes	7-D 7-D
ADP a7-19 (Rev.)	Detoxication mechanisms in cattle and sheep	Kerrville, Texas	Yes	7-E
ADP a7-20 (Rev.)	Characterization of cytological responses to toxic actions of pesticides and other agricultural chemicals in livestock and poultry	Kerrville, Texas	Yes	7-F
ADP a7-22	Studies of the incidence and pathology of cancer and other tumors in food-producing animals	Ames, Iowa	No	
ADP a7-23	Toxicological and pathological effects of insecticides, herbicides, fungicides, and other agricultural chemicals on livestock and poultry	Kerrville, Texas College Station, Texas	Yes Yes	7-G
ADP a7-24	Mycotic diseases of domestic animals	Ames, Iowa	Yes	7-H
ADP a7-25	Investigations of the genus Pasteurella	Ames, Iowa	Yes	5-D and 7-I
ADP a7-26	Biological changes associated with neuropathological conditions in animals *	Ames, Iowa	No	
ADP a7-27	Physiopathological Investigations of the interrelations between the respiratory, circulatory, and digestive systems of animals *	Ames, Iowa	No	
ADP a7-28	Proteins and other complex molecules from animal disease agents derived primarily from surface structures and extracellular products	Ames, Iowa	No	
ADP a7-29	Chemical and physical studies on microbial antigens	Ames, Iowa	Yes	7-I
ADP a7-30	Microbiology of the ruminant digestive tract and its relation to digestive disturbances	Ames, Iowa	Yes	7-J
ADP a7-31	Physiology of normal mammalian cells grown in tissue cultures	Ames, Iowa	No	

Area No. 7 - Cont'd.

Line Project Check List - Reporting Year July 1, 1965 to June 30, 1966

Work and Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Inc. in Summary of Progress (Yes-No)	Area and Subheading
ADP a7-32	Metabolic, antigenic, and pathogenic characteristics of Dermatophilus congolensis **	Ames, Iowa	Yes	7-K
ADP a7-33	Delineation of motor centers in the brain that are associated with motility of the ruminant esophagus and stomach **	Ames, Iowa	Yes	7-L
ADP a7-34	Physiological fate of rumen gases absorbed from the lungs following eructation **	Ames, Iowa	Yes	7-M
ADP a7-35	Correlation of the ultrastructural and biological properties of animal pathogens **	Ames, Iowa	Yes	7-N
ADP a7-36	The effects of mycotoxins on animals **	Ames, Iowa	No	
ADP a7-37	Relationship between psittacosis-group agents found in wild and domestic birds and domestic mammals **	Ames, Iowa	Yes	7-O
ADP a7-38	Teratogenic and toxic compounds from poison plants **	Logan, Utah	Yes	7-P
ADP a7-39	The role of parathyroid hormone and thyrocalcitonin in calcium metabolism **	Ames, Iowa	Yes	7-Q
ADP a7-40	Studies of pituitary-adrenal function in cattle **	Ames, Iowa	Yes	7-R
ADP a7-41	The toxicological effects of loco plants on livestock **	Logan, Utah	Yes	7-S
ADP a7-42	Development and modification of equipment for greater laboratory and animal room safety **	Ames, Iowa	No	
ADP a7-43	The role of physical, chemical, and biological aerosols in domestic animal diseases **	Ames, Iowa	No	

* Discontinued during reporting period.

** Initiated during reporting period.

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Inc. in Summary of Progress (Yes-No)	Area and Subheading
ADP a8	Foot-and-Mouth and Other Exotic Diseases of Cattle			
ADP a8-8 (Rev.)	Immunological investigations - Studies on foot-and-mouth disease virus	Greenport, N. Y.	Yes	8-A
ADP a8-10 (Rev.)	Immunological investigations to determine the mechanism of antibody formation using viruses of exotic animal diseases	Greenport, N. Y.	Yes	8-B
ADP a8-11 (Rev.)	Immune response to various types and subtypes of foot-and-mouth disease virus *	Greenport, N. Y.	No	
ADP a8-12 (Rev.)	Development of methods for production of large quantities of foot-and-mouth disease virus by tissue culture methods	Greenport, N. Y.	Yes	8-C
ADP a8-14 (Rev.)	Establishment and characterization of cell lines and cell strains for the propagation of foot-and-mouth and other exotic disease agents of cattle	Greenport, N. Y.	Yes	8-D
ADP a8-17 (Rev.)	Mechanism of the interaction between foot-and-mouth disease virus molecules and other exotic viruses with their host cells	Greenport, N. Y.	Yes	8-E
ADP a8-18 (Rev.)	Investigations of the genetic biochemistry of foot-and-mouth disease virus	Greenport, N. Y.	Yes	8-F
ADP a8-19 (Rev.)	Effects of certain chemical and physical environments on foot-mouth-disease virus	Greenport, N. Y.	Yes	8-G
ADP a8-20 (Rev.)	Bulk freeze-drying of foot-and-mouth disease virus, vaccines, and antiserums	Greenport, N. Y.	Yes	8-H
ADP a8-25	Identification, purification, and chemical and physical characterization of foot-and-mouth disease virus and other exotic animal viruses	Greenport, N. Y.	Yes	8-I
ADP a8-26	Immuno-chemical investigations of foot-and-mouth disease	Greenport, N. Y.	Yes	8-J
ADP a8-27	Microbiological investigations - Attenuation of representative types of foot-and-mouth disease virus	Greenport, N. Y.	Yes	8-K

Area No.8- Cont'd.

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Inc. in Summary of Progress (Yes-No)	Area and Subheading
ADP a8-28	Survival and inactivation of foot-and-mouth disease virus in meat and meat by-products *	Greenport, N. Y.	Yes	8-L
ADP a8-29	Studies on the biological mechanisms of natural resistance and susceptibility of foot-and-mouth disease virus	Greenport, N. Y.	Yes	8-M
ADP a8-30	Biological alterations of foot-and-mouth disease virus from continued residence in cell cultures	Greenport, N. Y.	Yes	8-N
ADP a8-31	Morphologic aspects of virus-cell relationships	Greenport, N. Y.	Yes	8-O
ADP a8-32	Diagnostic and immunizing procedures for contagious bovine pleuro-pneumonia	Greenport, N. Y.	Yes	8-P
PL 480 E3-ADP2	Studies on foot-and-mouth disease	Sao Paulo, Brazil	Yes	8-Q
PL 480 A22-ADP-8	Studies of various indigenous types of foot-and-mouth disease virus, and the production of a vaccine for the control of FMD in Turkey	Etlik, Turkey	Yes	8-R

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Summary of Progress (Yes-No)	Inc. in Area and Subheading
ADP a9	Foot-and-Mouth and Other Exotic Diseases of Swine			
ADP a9-1(Rev.)	Immunological investigations of foot-and-mouth disease of swine	Greenport, L. I., New York	Yes	9-A
ADP a9-2(Rev.)	Investigations of African swine fever	Greenport, L. I., New York	Yes	9-B
		Kenya, East Africa	Yes	9-B
PL 480				
E25-ADP-4	Diagnostic methods for African swine fever	Madrid, Spain	Yes	9-B

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Work and Line Project Number	Work and Line Project Titles	During Past Year	Line Project Inc. in Summary of Progress (Yes-No)	Area and Subheading
ADP all	Foot-and-Mouth and Other Exotic Diseases of Sheep			
ADP all-1	Immunological investigations of foot-and-mouth disease in sheep	Greenport, L.I., New York	Yes	10-A
PL 480				
A22-ADP-6	Preparation of a vaccine against sheep pox from tissue culture propagated virus	Ankara, Turkey	Yes	10-B
A7-ADP-5	Vaccine for protecting sheep against sheep pox	Madras, India	Yes	10-B

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Summary of Progress (Yes-No)	Inc. in Area and Subheading
ADP b1	Parasites and Parasitic Diseases of Cattle			
ADP b1-19 (Rev.)	Acquisition and effects of roundworm parasites of cattle as influenced by diet *	Beltsville, Md.	No	
ADP b1-23 (Rev.)	Host-parasite relationship of coccidial parasites of cattle	Auburn, Ala.	Yes	11-A
ADP b1-24	Ecology and immunology of the cattle lungworm, Dictyocaulus viviparus *	Beltsville, Md.	No	
ADP b1-25 (Rev.)	Clinical and physiological aspects of roundworm parasitism in cattle including anthelmintic	Davis, Calif.	Yes	11-B
ADP b1-26	Investigations of Trichomonad parasites	Logan, Utah	Yes	11-C
ADP b1-27	Host-parasite relationship of intestinal worms, Cooperia species, in cattle	Auburn, Alabama	Yes	11-D
ADP b1-28	Epizootiological-ecological investigations of the internal parasites of grazing cattle	Beltsville, Md.	Yes	11-E
ADP b1-29	Etiology and immune response of cattle to winter coccidiosis	Logan, Utah	Yes	11-F
ADP b1-30	Anaplasmosis of cattle	Beltsville, Md.	Yes	11-G
ADP b1-31	Interrelationship of diet and parasitic infection in the production of cattle	Auburn, Ala.	No	
ADP b1-32	Histochemistry of gastrointestinal nematodes of cattle *	Auburn, Ala.	Yes	11-H
ADP b1-33	Parasites of cattle, with emphasis on Stephanofilarial species	University Park, N. M.	Yes	11-I
ADP b1-34	Effect of stocking rate and rotational grazing on internal parasitism of cattle	Auburn, Ala. Experiment, Ga.	Yes Yes	11-J 11-J
ADP b1-35	Effect of host diet on the bionomics of the preparasitic stages of nematodes in cattle feces **	Auburn, Ala. Experiment, Ga.	Yes Yes	11-K 11-K
ADP b1-36	Life history and host-parasite relationships of Trichostrongylus affinis, a nematode parasite of rabbits **	Auburn, Ala. State College, Miss.	Yes Yes	11-L 11-L
ADP b1-37	Effects of level, rate, and period of exposure to larvae on the establishment and pathogenesis of gastrointestinal nematode parasites of cattle **	Beltsville, Md.	Yes	11-E
PL 480 S8-ADP-1	Environmental factors influencing parasites and parasitic diseases of economical importance in ruminants (cattle, sheep, and alpacas)	Lima, Peru	Yes	11-M
PL 480 S9-ADP-1	Anaplasmosis, piroplasmosis, and babesiellosis of cattle	Montevideo, Uruguay	Yes	11-G

* Discontinued during reporting period.
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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Inc. in Summary of Progress (Yes-No)	Area and Subheading
ADP b2	Parasites and Parasitic Diseases of Swine			
ADP b2-12 (Rev.)	Investigations of the swine intestinal roundworm, Ascaris suum	Lincoln, Neb.	Yes	12-A
ADP b2-15 (Rev.)	Investigations of strains of Trichinella spiralis resistant to heat and cold and modes of transmission of the parasite	Beltsville, Md.	Yes	12-B
ADP b2-17	Studies of Strongyloides ransomi infections in baby pigs	Tifton, Ga.	Yes	12-C
ADP b2-18	Evaluation of biochemical and other aspects of the host-parasite relationship in the development and severity of helminthiases of swine	Beltsville, Md.	Yes	12-D
ADP b2-19	Life cycle of the short-tail nodular worm of swine, Oesophagostomum brevicaudum *	Tifton, Ga.	Yes	12-E
ADP b2-20	Factors involved in the infection of the dung beetle, Phaneaus vindex, with the larvae of the thick stomach worms of swine *	Tifton, Ga.	Yes	12-F
ADP b2-21	Biology and control of Stephanurus dentatus, the swine kidneyworm *	Raleigh, N. C.	Yes	12-G
PL 480 E21-ADP-9	Trichinellosis with special reference to epizootiology, immunology, and pathogenesis	Warsaw, Poland	Yes	12-B

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Summary of Progress (Yes-No)	Inc. in Area and Subheading
ADP b3	Parasites and Parasitic Diseases of Sheep and Goats.			
ADP b3-16 (Rev.)	Gastrointestinal nematodes and nematodiasis of sheep and measures for their control.	Beltsville, Maryland Lexington, Kentucky	Yes Yes	13-B 13-B
ADP b3-18	The life histories, biology, pathogenesis and control of several helminth parasites of sheep occurring in the Southwest.	University Park, New Mexico	Yes	13-D
ADP b3-19	Studies on the life cycles of Eimeria ahsata and E. crandallis, pathogenic coccidia of sheep.	Auburn, Alabama	Yes	13-A
ADP b3-20	The effect of gastrointestinal nematodes on the tensile strength and sulfur content of wool.	Fargo, North Dakota	Yes	13-E
ADP b3-21	Immunity to the intestinal worm, Trichostrongylus colubriformis, a parasite of ruminants.*	Auburn, Alabama	Yes	13-C
ADP b3-22	Control of the common sheep scab mite, Psoroptes ovis.	Albuquerque, New Mexico	Yes	13-F
ADP b3-23	Chemical control of Oestrus ovis in sheep.**	Albuquerque, New Mexico	Yes	13-G
ADP b3-24	Biology and control of Psorergates ovis, the Australian itch mite of sheep.**	Albuquerque, New Mexico	Yes	13-H
ADP b3-25	Pathobiology of several laboratory and field strains of Psoroptes ovis, the mite of common sheep scab.**	Albuquerque, New Mexico	Yes	13-I
ADP b3-26	Overwinter survival of parasitic nematode larvae on Mississippi pastures.**	Auburn, Alabama State College, Mississippi	Yes	13-J
ADP b3-27	The biology and control of the liver fluke, Fasciola hepatica, in the Southwest.**	University Park, New Mexico	Yes	13-K
PL 480				
A22-ADP-1	Transmission, distribution, and bioeconomics of the giant liver fluke of domestic ruminants in Turkey.*	Ankara, Turkey	Yes	13-K

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Inc. in Summary of Progress (Yes-No)	Area and Subheading
ADP b4	Parasites and Parasitic Diseases of Poultry			
ADP b4-9	Investigations for controlling coccidiosis of poultry.	Beltsville, Maryland	No	
ADP b4-10	The biology of the nematode parasite of poultry and related birds with special reference to the application of findings to control measures.	Beltsville, Maryland	No	
ADP b4-11	Biological investigations of protozoan parasites and parasitic diseases of poultry, with special reference to those of the gastrointestinal tract.	College Station, Texas Beltsville, Maryland	Yes Yes	14-A 14-A
PL 480 A6-ADP-1	Leucocytozoon infection in chickens and development of effective treatment.	Taipei, Taiwan, China	Yes	14-A

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Inc. in Summary of Progress (Yes-No)	Line Project Inc. in Area and Subheading
ADP b5	Treatments for Removal or Control of Parasites of Domestic Animals			
ADP b5-16	Control of internal parasites of livestock by management practices that will not create consumer residue hazards *	Auburn, Ala. Experiment, Ga.	Yes Yes	15-A 15-A
ADP b5-17	Investigations of antiparasitic agents and measures for the control of parasites belonging to the family Oestridae	Albuquerque, N.M.	No	
ADP b5-18	Investigations to develop new and improved chemical agents for the treatment, prevention, or control of helminthic parasites in farm animals	Beltsville, Md.	Yes	15-B
ADP b5-19	Potential chemotherapeutic agents as treatments for bovine venereal trichomoniasis	Beltsville, Md.	Yes	15-C
ADP b5-20	Evaluation, development and standardization of antiparasitics of established, or reported value **	Beltsville, Md. Auburn, Ala. Experiment, Ga. State College, Miss.	Yes Yes Yes Yes	15-D 15-D 15-D 15-D
ADP b5-21	Control of lice on cattle	Albuquerque, N.M.	Yes	15-E

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Project Inc. in Summary of Progress (Yes-No)	Area and Subheading
ADP b6	Miscellaneous Parasites and Parasitic Diseases			
ADP b6-9 (Rev.)	Publication of author, subject (parasite) and host index-catalogues of medical and veterinary zoology	Beltsville, Md.	Yes	16-A
ADP b6-10 (Rev.)	Investigation of immunologic and other biologic approaches to the prevention and control of parasitic diseases	Beltsville, Md. St. Paul, Minn. Madison, Wisc.	Yes Yes Yes	16-B 16-B 16-B
ADP b6-11	Studies of the chemical and physical lements of parasites and parasite-host relationships in animals	Beltsville, Md.	Yes	16-C
ADP b6-12	Taxonomic investigations of helminths and other parasites	Beltsville, Md.	Yes	16-D
ADP b6-13 (C)	Equine piroplasmosis	Beltsville, Md. Gainesville, Fla. Lexington, Ky.	Yes Yes Yes	4-A 4-A 4-A
ADP b6-14	Maintenance of author, parasite-subject, host, and anthelmintic catalogues and checklist of specific and subspecific names	Beltsville, Md.	Yes	16-A
ADP b6-15	Maintenance of parasite collections	Beltsville, Md.	Yes	16-E
ADP b6-16	Identification of parasites of importance in parasitological research, regulatory, and quarantine, and other work	Beltsville, Md.	Yes	16-D
ADP b6-17	Pigments of parasites *	Beltsville, Md.	Yes	16-F
ADP b6-18	Biology, epidemiology, and pathogenicity of demodectic mange of domestic livestock *	Albuquerque, N.M.	Yes	16-G
ADP b6-19	Cytological investigation of protozoan parasites that penetrate the gastro-intestinal tract of poultry and other farm animals *	Beltsville, Md.	Yes	16-H

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